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A Summary of Current Program and
Preliminary Report of Progress

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CROSS COMMODITY RESEARCH OF AGRICULTURAL ENGINEERING,
CROPS, AND ENTOMOLOGY RESEARCH DIVISIONS

of the

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

This progress report of U.S.D.A. and cooperative research is primarily a tool for use of scientists and administrators in program coordination, development and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of progress on U.S.D.A. and cooperative research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members and others having an interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of U.S.D.A. and cooperative research issued during the past year. Current agricultural research findings are also published in the monthly U.S.D.A. publication, Agricultural Research.

UNITED STATES DEPARTMENT OF AGRICULTURE
Washington, D. C.
December 31, 1963

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ADVISORY COMMITTEES

The research program of the Department of Agriculture is reviewed annually by the following advisory committees:

1. Farm Resources Research
2. Utilization Research and Development
3. Human Nutrition and Consumer Use Research
4. Marketing Research and Service
5. Agricultural Economics Research
6. Forestry Research
7. Animal and Animal Products Research
8. Cotton and Tobacco Research
9. Grain and Forage Crops Research
10. Horticultural Crops Research
11. Oilseed, Peanut and Sugar Crops Research

ORGANIZATIONAL UNIT PROGRESS REPORTS

Source materials used by the advisory committees are of two types. First there are organizational unit reports that cover the work of the Divisions or Services listed below. The number prefixes refer to advisory committees listed above that review all of the work of the respective Divisions or Services.

Agricultural Research Service (ARS)

- 1 - Soil and Water Conservation
- 2 - Utilization -- Eastern
- 2 - Utilization -- Northern
- 2 - Utilization -- Southern
- 2 - Utilization -- Western
- 3 - Human Nutrition
- 3 - Clothing and Housing
- 3 - Consumer and Food Economics
- 7 - Animal Disease and Parasite
- 7 - Animal Husbandry

Agricultural Marketing Service (AMS)

- 4 - Market Quality
- 4 - Transportation and Facilities

Economic Research Service (ERS)

- 4,5 - Marketing Economics
- 5 - Farm Production Economics
- 5 - Resource Development Economics
- 5 - Economic & Statistical Analysis
- 5 - Foreign Development and Trade Analysis
- 5 - Foreign Regional Analysis

Other Services

- 1 - Soil Conservation Service (SCS)
- 4,5 - Farmer Cooperative Service (FCS)
- 4,5 - Statistical Reporting Service (SRS)
- 6 - Forest Service (FS)

Three organizational unit reports are not reviewed in entirety by any one committee. All of the information in them is included in the subject matter reports.

Agricultural Research Service (ARS)

Agricultural Engineering
Crops
Entomology

SUBJECT MATTER PROGRESS REPORTS

The second type of report brings together the U.S.D.A. program and progress for the following commodities and subjects:

- | | |
|--|--|
| 1 - Cross Commodity Research of
Agricultural Engineering, Crops,
and Entomology Research Divisions | 7 - Cross Species and Miscellaneous
Animal Research |
| 3 - Rural Dwellings | 8 - Cotton and Cottonseed |
| 6 - Forestry (Other than Forest
Service) | 8 - Tobacco |
| 7 - Beef Cattle | 9 - Grain and Forage Crops |
| 7 - Dairy | 10 - Citrus & Subtropical Fruit |
| 7 - Poultry | 10 - Deciduous Fruit & Tree Nut |
| 7 - Sheep and Wool | 10 - Potato |
| 7 - Swine | 10 - Vegetable |
| | 10 - Florist, Nursery & Shade Tree |
| | 11 - Oilseeds and Peanut |
| | 11 - Sugar |

A copy of any of the reports may be requested from David J. Ward, Executive Secretary, Farm Resources Research Advisory Committee, Agricultural Research Service, U. S. Department of Agriculture, Washington, D. C.

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INTRODUCTION

This is a report of the Department's research that does not have a major commodity orientation. It deals primarily with the non-commodity research of the Agricultural Engineering, Crops, and Entomology Research Divisions of the Agricultural Research Service. This includes the basic research of several Pioneering Laboratories.

Under each of the Problem Areas listed in the report is a statement describing the program of work underway and the professional man years devoted to the major kinds of research included.

A brief report of progress and significant findings is given for each part of the research program.

A large part of the Department's research is cooperative with State Experiment Stations. Many Department employees are located at State Stations and use laboratory and office space close to or furnished by the station. Cooperative work is jointly planned, frequently with the representatives of the producers or industry affected participating. The nature of cooperation varies with each study. It is developed so as to fully utilize the personnel and other resources of the cooperators which frequently includes resources contributed by the interested producers or industry.

The following are some examples of recent accomplishments of cooperative U.S.D.A. research of the sort described in this report:

Soil-machine relationships. Determinations of the effects of plow size and type of performance in different soil types and conditions; tire characteristics such as cord arrangement, tread design, rim width and diameter, and inflation pressure on the performance of traction tires on different soil types and field conditions; and methods of manufacture and steel specifications on the service of disks used on agricultural implements are being used extensively by the farm equipment industry and technical advisors to farmers as well as directly by farmers.

Purification and separation of plant viruses. A new procedure has been developed for purification and sorting of viruses, proteins, and cellular fragments according to their particle size. This procedure involves use of appropriate buffers to prevent aggregation and denaturation

of the desired components, activated charcoal for the removal of color pigments, and columns of granulated agar gel for removal of undesirable salts and sorting of particles on the basis of size. The procedure is very useful in preparation of monodispersed suspensions of large molecules, viruses, and small cellular components. For chemical, physical, serological, or biological studies of these materials, it is often very important that one work with suspensions of highly purified material. Even small amounts of impurities often result in erroneous observations. Therefore, any procedure that improves on methods already available is exceedingly important.

Sterility method of insect control. A new concept in controlling insects and other pests has been developed which may contribute to the solution of some major insect problems. The method involves the release of sterile insects for their own destruction. The mass production and release of screw-worm flies made sterile by gamma radiation resulted in the elimination of this important livestock pest from the Southeast. A similar and more complex program based on the same principle which is now underway in the Southwest has already greatly reduced screw-worm infestation in the area. The utilization of sterile insects was responsible for the eradication of the melon fly from the South Pacific island of Rota, and the method is being intensively investigated as an aid to the control or eradication of other major pests, including the boll weevil, pink bollworm, sugarcane borer, codling moth, tobacco hornworm, and other pests. An important advance in research on the sterility method has been the development of several chemicals which produce sterility in insects with less damage to the insects than that caused by radiation. The availability of such chemicals also offers the possibility of discovering ways to induce sterility in the natural insect population, thus obviating the necessity of rearing and releasing insects sterilized by radiation to achieve control.

SOIL - MACHINE RELATIONSHIPS
Agricultural Engineering Research Division, ARS

Problem. The substitution of the internal combustion engine for animal power has been the major influence on the farmer's productivity during the first half of the twentieth century. There have been important developments in the tractor chassis and its accessories, such as tricycle gear, power take-off, implement mounting, hydraulic controls, and pneumatic tires, but there is still a lack of fundamental knowledge and understanding of the method whereby tires and tracks transmit forces to the soil in developing traction. In view of the tremendous amount of power and energy which is used every year in farm field operations, all factors which may affect the efficiency of this use should be continually studied for potential improvements in efficiency.

There is need for basic information on how traction is developed by tires and tracks, and need for improved traction, and transport equipment. There is evidence that compaction of soils is becoming more common because of the increasing size of tractors and the more complete mechanization of field operations, particularly harvesting, which usually must be done at a given date regardless of the soil conditions; thus, associated with tire and track research is a need for study of methods of reducing soil compaction.

Tillage of the soil is the greatest consumer of power in the production of crops in the United States today. Some type of tillage operation is considered necessary prior to the growing of almost all crops. Despite this great need and cost, the tillage tools which are generally used have remained essentially unchanged since their invention, or most radical improvement, nearly 100 years ago, and very few innovations since have survived the tests of improved crops response and/or reduced cost of operation. While some tillage is needed for nearly all crops, there is good evidence that much unneeded and in some cases detrimental tillage operations are performed. The soil is a very complex physical system, containing inorganic and organic solids, liquids and gases, and its reactions to forces, manipulation, temperature, and water is unlike any other simple material. In view of the wide-spread use of, and great power consumption by, tillage, there is a need for expanded basic research to give more precise information on the inter-relationship of tillage, soil physical conditions, and plant growth; on the effect of soil mechanics upon the tillage operation; on the effect of equipment mechanics on the tillage operation; on mathematical methods which can be used to predict the effect of various forces on the soil; and on tillage methods and systems of equipment which are compatible with conservation farming practices. Intensive research is needed to determine the optimum tillage requirements, based on costs and crop response, for various soil, climatic and crop conditions.

USDA PROGRAM

The Department has a continuing long-term program involving agricultural engineers and soil scientists engaged in both basic studies and the application of known principles to solve problems dealing with the relationships between soil-engaging equipment and soil reactions. The research findings are applicable to tillage implements, tractive and transport equipment (such as tires, wheels, and crawler tractor tracks), and soil moving equipment (such as land forming and road building equipment). Work is cooperative with the State Agricultural Experiment Stations at Auburn, Alabama; Ames, Iowa; Athens, Georgia; State College, Mississippi; and East Lansing, Michigan. USDA personnel working on this project are stationed at Auburn, Alabama, and Ames, Iowa. Much of the work at the laboratory at Auburn is with manufacturers of implements and equipment for use in agriculture. The research is of a fundamental nature of value to the entire industry and directly and indirectly to farmers. It consists of theoretical analyses, basic laboratory studies, controlled soil bin tests, and field observations.

The Federal scientific effort devoted to research in this area totals 7.3 professional man-years. Of this number 1.0 is devoted to traction and transport devices and soil reaction; 1.0 to the effect of tillage practices on plant growth; 1.2 to the measurement of soil physical properties; 1.5 to equipment mechanics; 0.5 to the effect of soil mechanics; 0.5 to methods of mathematical analysis; 1.0 to systems of equipment for conservation farming; and 0.6 for program leadership.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

An important development in this field of research was the dedication of new facilities at the National Tillage Machinery Laboratory at Auburn, Alabama on May 17, 1963. A new building for tillage research has 14,000 square feet of floor space, and provides two 20 X 200-foot soil bins that permit year-round operations. These two new bins will supplement the nine similar-sized outdoor soil bins which are being used in tillage research at Auburn. Also dedicated was a new laboratory-administration building, which houses three laboratories, service facilities, and offices. Scientists in two of the laboratories will study physical and mechanical properties of soils and how the physical condition of soil affects plant growth. In the third laboratory, model tillage tools will be tested. In dedicating this new building, Dr. Byron T. Shaw, Administrator of ARS said: "We hope, with the increased emphasis on the basic work here to find some of the answers to farmers' tillage problems, and to develop principles that can be used to help farmers fully utilize their equipment".

A. Traction and Transport Devices and Soil Reaction.

This project is designed to determine and evaluate the effects of various construction, material, and operational factors on the

performance of tires and tracks when used for traction and for transport.

1. A study designed to determine the effect of rim diameter on rear tractor tires showed that drawbar pull, coefficient of traction, and power efficiency all increased with increases in rim diameter. All tires had the same cross sectional area, lug design, were loaded with the same static load, and inflated to give the same deflection. Performance of all tires were compared in the same soil conditions and two soil types. Five 12.4" tires with rims varying from 24" to 42" were used. In Lloyd clay the tire on the 42" rim pulled as much at 16% slip as the tire on the 24" rim pulled at 26% slip and 25% more with both tires slipping 16%. The differences were approximately the same in a loam soil. Loading the tires to give them equal dynamic weight at 20% slip reduced the differences only slightly.

Another phase of this study was to test two tires having equal static load ratings but varying in both tire section and rim diameter to determine whether or not increasing tire width would compensate for the increase in rim diameter. Tires used were a 12-38 and 13-28 both inflated to 14 psi. At 16% slip the increase in drawbar pull for the 38" rim was 10% and 20% respectively in the clay and loam soil. This is near the differences found for the 12.4" tires having 38 and 28 inch rims respectively.

Cooperation with the ASAE Tractive and Transport Efficiency Committee was continued. Two programs are underway. Progress is being made on the development of a glossary of terms. The second program, recently organized, is designed to determine the effects of track shoe design on track performance. Preliminary work is underway on this study.

Two series of tests were made comparing conventional and low pressure tires as traction devices. Data for these tests are not completely summarized. Final reports will be prepared as quickly as possible.

Preliminary tests have been made cooperatively with a group representing companies manufacturing rear tractor tires in an attempt to develop a basis for giving tires a torque rating comparable to the static load rating now used. This work will be continued.

A program to determine the effects of lug stiffness on the performance of rear tractor tires was started. Tests data indicate that the first tires prepared for use in this study did not have needed variation.

The bar-table developed on this project and used in the studies of tractor tire tread movement was loaned to one of the tire companies. They report that it was an effective tool in helping to determine the effects of some tire designs on tire wear.

B. Effect of Tillage Practices on Plant Growth.

1. The study of basic factors applicable to the design and use of deep tillage implements was continued. The 18" vs. 6" depth of tillage plots were ripped 6" deep over the row only and planted to determine any carry-over effect of the deep tillage treatment. The yields of cotton were 1.36 bales/acre for deep tillage (18") and 1.31 bales/acre for the check treatment (6"). The yields of cotton on the 36" vs. 6" depth of tillage plots were 1.56, 1.44, and 1.41 bales/acre respectively for the 36" depth of tillage 36" fertilizer depth, 36" depth of tillage 6" fertilizer depth, and 6" depth of tillage 6" fertilizer depth. The rooting depth of the main taproots were 12.27", 11.40", and 8.35" respectively. A good stand was not obtained on the plots this year which partially accounts for the lower yields. After the 1963 season it is anticipated that these plots in the Lloyd clay loam will be discontinued and similar trench tillage plots will be established on Norfolk sandy loam.

C. Measurement of Soil Physical Properties.

1. This project covers the work on the relationship of soil physical conditions to design and use of tillage machinery. It includes basic laboratory studies, bin tests, and field tests.

Theoretical terms that have been used successfully in similitude studies of plane chisels in artificial soil were employed in similar studies with natural soil. Model data predicted prototype results satisfactorily but not as closely as they did in artificial soils. Cause of this deviation can be attributed to soil cones forming on the tools only in natural soil. The effect of these soil cones was to essentially alter the shape of the tool beyond that which was accounted for in the assumed pertinent variables.

Experimental work completed in a deeper spring and fall plowing study was initiated. Significant reductions in soil strength as measured by the penetrometer have been secured by deeper plowing which removed a traffic pan. Yield data did not reflect any difference due to method of tillage but moisture was not a limiting factor in this crop year. While there was a slightly greater cloddiness in the deep plowed plots, there was no reduction in cotton stands.

Tensile strength studies were continued with prepared briquettes made from the Laboratory soils. Strength-moisture loss relationships were continued on a limited basis and no new conclusions can be reached concerning this aspect of work.

This was the fourth year of a 5 to 7 year planned program to determine the effects of the crop preceding and the depth of a seedbed preparation on peanut production.

The entire area was plowed uniformly in 1962 and planted to the selected variety of crops that precede the 1963 peanut crop.

Wear resistance data of plastic materials was analyzed from laboratory tests of different size abrasive sand. The coarser sand particles caused a greater difference in wear resistance between polytetrafluoroethylene (Teflon) and polyethylene. A new commercial wear machine for plastics was tried but found to have less correlation with field experience than the National Tillage Machinery Laboratory wear machine. Field studies of wear resistance of plastics with farmers was continued which showed both Teflon and polyethylene economically feasible for use.

D. Equipment Mechanics.

1. The basic studies of disk design and operating parameters have been continued with full scale tools. The completion of a new dynamometer unit permitted a more accurate series of measurements. A disk angle control device was developed so that the angle about the vertical axis could be remotely controlled and its value recorded during operation. A series of studies was designed and measurements made to determine the influence of the disk angle on the forces which the soil applied to the disk during operation. The accuracy and completeness of the data was considerably better than that which was secured with the old apparatus. Continuous plots of disk angle vs. draft relationships were secured for five different soil conditions. Lateral and vertical soil forces on the disks were also measured as well as the moments associated with the three forces. Soil forces on the back side of the disk were greatest at low disk angles and in hard soil. The maximum net lateral force on the back of a 26 inch disk operating at a depth of 5" and taking a 6" width of cut was 360 pounds. This force was reduced to zero at approximately 40 degrees. The net force on the front of the disk then increased to 40 pounds at 50 degrees. Thus the technique of programming the angle of cut permits a rapid method of determining the angle at which the lateral forces are balanced on disks.

E. Effect of Soil Mechanics.

1. This research is designed to determine and evaluate the effects of various machine loads on the compaction of soil. Stress measurements and bulk density measurements made under single, dual and Terra (wide low profile) tires were made. Some attempts were made to improve prediction formulas but no significant improvement can be reported at this time.

F. Methods of Mathematical Analysis.

1. No new data were acquired during the past year. Further analysis of triaxial data reported last year indicates that one fact is clearly established. Soil compaction (volume strain) cannot be uniquely related to the magnitude of applied forces. Rather, compaction is related to the

magnitude of the forces and also the manner in which they are applied. The effect of manner, however, may be small enough so that in some circumstances it may be ignored since the most extreme manner of loading produced a difference of less than 10%. A thorough review of the mechanics of a continuous medium was undertaken in connection with a graduate course at Auburn University. Several possible mathematical strain descriptions were evaluated in an attempt to find suitable representation for large strains that often occur in soil.

G. Systems of Equipment for Conservation Farming.

1. The determination of requirements of tillage for corn was continued in Iowa with emphasis on the selection of suitable soil constants, methods of measurement and establishment of critical limits for both the row and interrow zones. Using the point quadrant technique and a self-recording automatic soil profile meter developed, changes in the porosity of the tilled soil layer and surface microrelief resulting from tillage operations were discernible among different tillage systems. Both total porosity and surface roughness are relevant to water management in the interrow area and are useful in evaluating tillage systems. A study was initiated to characterize changes in other properties of the plow layer attributable to tillage implements. Information of this nature will lead to the design and development of more effective and efficient tillage implements and systems.

A rotary sieve was constructed for measuring clod size distribution in soils. Investigations relative to the effect of aggregate size on their properties were continued, with emphasis on the moisture retention and transmission characteristics and organic matter distribution within aggregates. The volume of water retained per unit volume of aggregate decreased as aggregate size increased and was associated with changes in apparent density of the aggregate. In most soils studied the carbon and nitrogen content of aggregates was inversely related to aggregate size. Generally the carbon and nitrogen content of the surface of an aggregate were greater than that within the aggregate. Soil aggregates are discrete entities possessing moisture and organic matter characteristics that vary with size. Such information is useful in evaluating the row zone resulting from various tillage practices. Major corn yield differences due to tillage practice at 5 locations in Iowa were generally related to difference in stand.

Early spring applications of herbicides to control weeds made it possible to successfully grow corn following corn without tillage. Surface residues caused some difficulty in planter operation, but tilling a narrow band at a shallow depth greatly reduced this problem. Manipulating the soil with tillage tools may be unnecessary if weeds are controlled chemically and satisfactory stands are obtained. However, on some soils soil disturbance to break the surface crust may be desirable for soil and

water conservation. Yield results of continuous corn on contoured unplowed ridges showed that after 11 years high yields (100 bu./A.) were maintained with practically no erosion. Results of model studies on the use of "Teflon" on the surface of tillage tools showed that scouring was improved, draft was reduced 6 to 38 percent, and that "Teflon" or "Teflon" with glass filler would wear 8 to 10 times faster than steel.

H. Foreign Research Under Public Law 480 Funds

1. A 2-year research project was completed in May, 1963, under a contract under PL 480 funds by the State Research Institute of Agricultural Engineering, Helsinki, Finland. The research included studies of: characteristics of wheel-type farm tractors on steep slopes (such as cleared forest areas), gasoline and diesel engine performance at low temperatures, suitability of wheel-type farm tractors for forestry work, and the mechanization of forest tree seeding.

Tractors were tested on level ground and on 10 and 15° slopes in summer and winter, under various soil temperature, and moisture conditions, and wheel chains were developed for improving the pulling power of the tractor especially on slopes. Equipment used to improve the pulling power included: wheel weights, special "Vakola" wheel chains developed at the Station, half-tracks for use with the rear wheels, and dual wheels. On a meadow with 10° slope for example, the best drawbar pulls were obtained when the tractor was equipped with wheel weights, or when the tractor was equipped with both wheel weights and wheel chains. On a meadow with 15° slope, the best drawbar pull was obtained when the tractor was equipped with both half-tracks and wheel weights.

Winter tests were made on a level ice covered farm road cleared of snow, on a level road made by compacting the snow, and on an ice covered farm road cleared of snow with 10° slope. The best drawbar pulls on any of these winter roads were with the "Vakola" wheel chains designed at the Station, followed by the half-tracks, and then the snow chains. However, these chains, because of their spikes, are prohibited by law from public roads, and are not allowed on maintained winter roads at the large logging sites because the chains break the road surface. The Vakola wheel chains are considered suitable for work in the farm forest or small area.

Tests were made in Finland on a gasoline burning tractor engine to determine the effect of the following factors on the wear rate of the top piston ring: working temperature, oil viscosity, repeated cold starts, loading, engine speed, and additives. The wear tests on the piston rings were made by the use of radioactive isotopes. The top piston rings were irradiated in the Harwell pile in England, and were fitted to the engine by the use of long-handled tools. The radioactivity of the oil, measured after a certain period of time, furnished an indication of the wear of the piston rings. The results of the wear tests relating to the working temperature

of the engine showed that the wear rate was considerably higher at the lower temperatures, especially at starting, caused chiefly by lack of enough oil in the cylinders. The wear rate was lowest at 80° C (176°F). No significant effect on the wear rate was observed during the tests from the additives sprayed into the inlet manifold, or mixed with the fuel or with the oil. It is hoped that a report can be issued in English on the research conducted in Finland for use by our professional and technical workers that are interested in the performance of farm tractors in cold climates.

The suitability of wheel-type farm tractors for forestry work was investigated during two winters in Finland. Timber harvesting in that country is usually done in the wintertime. In large, state owned logging sites in North-Finland during the 1962-63 harvesting season, about 50 percent of the timber was transported by tractors, 10 percent by trucks, and 40 percent by horses.

Important factors when considering the suitability of farm tractors for this work included: travel speeds, drawbar performance, braking efficiency, and ability to maneuver in rough terrain. Travel speeds available (1 to 18 mph) were considered adequate except that 20 mph would be advantageous for idle travel on main haulage roads and highways. Drawbar horsepower for year-round timber transport should be somewhat more than on present common farm tractors - probably a wheel-type farm tractor weighing about 4 tons with a 70 to 80 hp engine would be more suitable to forest work. Braking efficiency of the tractor would have to be improved for taking timber out of the woods in hilly terrain. A ground clearance of about 2 feet would be necessary in the roadless terrain. Tractors used in timber transport should have power take-off provisions from the sides, front, and rear.

Tests were made of tillage implements in Finland for use in mechanizing ground preparation on steep slopes for the seeding and planting of trees. The condition desired for planting was about 650 "spots" per acre from which the raw humus was entirely removed without loosening the mineral soil, each spot about one square foot in area. Several of the implements tested made bare spots as they progressed up or down the slope, each spot usually varying from about one to three square feet in area, with average skips between spots of from 8 to 16 feet within the row, and with about 6.5 feet between rows of spots. A land clearing moldboard plow and a disk plow were also tested for comparison with the implements that made rows of spots cleared of humus.

Two wheel tractors were used for the tests which had been specially reinforced and shielded to prepare them for the severe service required. Each was also provided with a front bumper and ballast weighing about 550 lbs. A safety cab with a sheltering steel wire net was provided to protect the operator. Observations made during the tests included the time

for making a spot line, the length of spot line, thickness of humus, and the frequency, area, and depth of spots. The mean net work times per acre for all of the implements were: 1.14 hrs. on a steep stoneless slope, 1.31 hrs. on a steep slope with a thin layer of humus, 1.44 hrs. on a steep slope very stony, and 1.62 hrs. on a slightly inclined slope with a thick layer of humus.

2. A 4-year research project contract was executed December, 1961, under PL 480 funds with the Agricultural Research Station, Beit Dagan, Israel. The research conducted concerns tillage methods and implements for mountain farms. Field experiments have been conducted to study soil translocation, both qualitatively and quantitatively, as affected by tillage implements, tillage methods and slope.

The following treatments were examined:

- a. Plowing with a moldboard plow along the contour turning the furrow downhill.
- b. As above, but turning the furrow uphill.
- c. Plowing with a disc plow along the contours, turning the furrow uphill.
- d. Tillage along the contour with a cultivator and an orchard disc harrow.
- e. Tillage perpendicular to the contour with a moldboard plow and a cultivator.

The treatments were applied on one or more of the following gradients: 17-19%; 13-15%; 10-12%; and 5-9%.

The area chosen for the experiment was typical of the soils of the hilly regions, combining all the above gradients. It was mapped and a plot of 12 x 35 m (39 x 115 ft.) was allotted to each treatment on each gradient. Implement performance was registered during tillage, together with tillage depth, tillage width, speed, and qualitative evaluation of the performance. The extent of soil translocation was measured after each tillage and expressed as the changes occurring on the profile at three cross-sections in each of the tilled plots. The surface of the ground was determined with a "profilograph" that was specially designed for this purpose.

The following conclusions may be drawn from the first year's investigations: uphill casting of the furrow with a moldboard plow, working on the contour, prevented extreme downhill movement of the soil in gradients up to 19%. Similar results were obtained with a disc plow, but the maximal gradient was limited to 13% in this case. Tillage with a cultivator did not cause any soil shift on the steepest slopes up to 19%. Similar results were obtained with an orchard disc harrow but with a maximum slope of 9%. Plowing with a moldboard plow perpendicular to the contour resulted

in a slight downhill soil movement. Performance of various tillage implements on slopes exceeding 15% will be studied further.

PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

Traction and Transport Devices and Soil Reaction.

- Vanden Berg, G. E. and Gill, W. R. 1962. Pressure Distribution Between a Smooth Tire and the Soil. Transactions of ASAE, Vol. 5, No. 2, pp. 105-107.
- Forrest, P. J., Reed, I.F., and Constantakis, G. V. 1962. Tractive Characteristics of Radial-Ply Tires. Transactions of ASAE, Vol. 5, No. 2, pp. 108 and 115.
- Reed, I. F. 1962. Effects of Inflation Pressure, Load Drawbar Pull on Axle Height and Rolling Radius of Six Tires. Transactions of ASAE, Vol. 5, No. 2, pp. 125 and 132.
- Vanden Berg, G. E. and Reed, I. F. 1962. Tractive Performance of Radial-Ply and Conventional Tractor Tires. Transactions of ASAE, Vol. 5, No. 2, pp. 126-129 and 132.
- Wann, W. L. and Reed, I. F. 1962. Studies of Tractor Tire Tread Movement. Transactions of ASAE, Vol. 5, No. 2, pp. 130-132.
- Vanden Berg and Reed, I. F. 1962. Evaluating Performance of Traction Devices. Presented at National Farm Construction and Industrial Machinery Meeting of S.A.E., Milwaukee, Wis., Sept. 10 - 14.

CONSTRUCTION STANDARDS, WATER SUPPLY,
WASTES DISPOSAL AND FARMSTEAD PLANNING

Agricultural Engineering Research Division, ARS

Problem. The inventory value of farm buildings in the United States exceeds \$28 billion. During 1960, more than 1/2 billion dollars were spent on repair and maintenance of these buildings and an additional 1-1/2 billion was spent for new construction, additions, and major improvements. The construction of many of these buildings is wasteful of materials. The strength of structural members is frequently much greater than the joints that connect them. Assumed loads are sometimes unrealistically high and at other times so low that failures occur. Better information on the actual loads--contents, wind, and snow--is needed. The basic frames of farm structures are often statically indeterminate and defy the accurate application of conventional indeterminate analysis techniques. Two research approaches are needed to develop readily applicable design procedures--proof testing and development of basic design formulas. Rural fires account for 800 deaths and 175 million dollars worth of property damage annually. Information is needed on means of reducing these losses without imposing excessive expense on construction.

In many localities urban building codes that may be unduly restrictive are being extended to cover farms. The hazards of public occupancy and damage to the property of others are not present to the degree that they are in urban areas. Those who draft building and fire codes need design information that would be realistic for farms.

An adequate supply of satisfactory water is essential to the farmstead. Automatic running-water systems, more water-using equipment, new uses for water, higher standards of sanitation, and other factors are continually increasing the demand for water on the farmstead--both in quantity and quality. The "old well" is less and less able to satisfy the demand. Some farm operators have been forced to buy water by the tank or truck load at considerable cost; others are developing farm ponds as sources of farmstead water; some continue to operate with a supply that is becoming less and less adequate.

Surface waters normally require disinfection as a safeguard against water-borne diseases such as typhoid, dysentery, other gastro-intestinal disorders, and infectious hepatitis. Often they also require filtration and other treatment to remove undesirable foreign material. Deeper ground waters are often highly mineralized (hardness, iron, sulphur, and others), and expensive or impossible to treat adequately. Data on water demands and water systems requirements of the modern farmstead are needed to guide farmers in planning water systems and selecting equipment, to enable extension workers to adequately advise farmers, and to guide equipment and appliance manufacturers and sanitary code-making bodies. Simpler, more reliable, and less costly methods and equipment are needed for treating farmstead water supplies to improve their quality.

Disposal of organic wastes--principally sewage and manures--is becoming more and more of a problem on the modern farmstead. The cattle, hogs, horses, sheep, and poultry on farms in the United States produce more than 2 billion tons of manure annually. The problem is particularly acute with respect to confinement-type livestock operations on the fringe of metropolitan areas--where the total amount of manure is concentrated in the confinement area. Under these conditions it is difficult to avoid creating a sanitation hazard or a public nuisance. Economical, sanitary means of disposition need to be developed. Among means that need to be investigated are lagoons, irrigation systems, subsurface absorption systems and reclamation. Development of improved methods for disposing of sewage in those rural areas where conditions are adverse to the conventional septic tank system (high ground water, shallow rock, non-absorptive soils, restricted areas) is needed.

The arrangement plan of the farmstead has an important bearing on its efficiency, appearance, and livability. For example, convenient locations for feed and bedding storage ease the distribution chore. A 40-cow dairy herd will use approximately 240 tons of silage, 60 tons of grain, 40 tons of hay, and 20 tons of bedding annually. Research is needed to evaluate the various planning factors in the light of current equipment and practices and to develop planning principles and guidance materials for the benefit of farmers--particularly those contemplating changes.

A 1957 survey of about 25 well-informed leaders of the agricultural community in each state reported the following as the most urgent problems needing solution:

1. How to adapt existing buildings for more efficient production.
2. Lower cost, more efficient, and more flexible buildings for tomorrow.
3. Engineered farmstead design.
4. Mechanization of materials handling.
5. Better utilities--water supply, wastes disposal, and electric service.

USDA PROGRAM

This is a continuing long-term program involving engineers and architects engaged in basic and applied research on structural aspects of farm buildings, farmstead water supply, farmstead wastes disposal and farmstead planning. Protection of farm families and animals against radioactive fallout has recently become of concern. The program is cooperative with selected State Agricultural Experiment Stations.

A. Meteorological factors affecting the design of farm structures, such as climate and weather (wind, storms, frost, etc.), are studied at Beltsville, Md., and selected field locations. A contract let to the Weather Bureau, USDC, for data on snow load probabilities, was completed during the year.

B. Construction standards, such as serviceability and safety, for design of farm buildings are studied at Beltsville, Md., and selected field locations. Liaison is maintained with the American Society of Agricultural Engineers, American Standards Association, National Safety Council, National Fire Prevention Association, and other organizations concerned with standards and safety in farm structures.

C. Materials and construction methods for farm buildings are studied at Beltsville, Md.; at Blacksburg, Va., in cooperation with the Virginia Agricultural Experiment Station; and at State College, Miss., in cooperation with Animal Husbandry Research Division and the Mississippi Agricultural Experiment Station.

D. Water supply and wastes disposal for the farmstead are studied at College Park, Md., in cooperation with the Maryland Agricultural Experiment Station. Liaison is maintained with the Public Health Service, the Water Systems Council, American Society of Agricultural Engineers, and other organizations concerned with rural sanitation.

E. Farmstead planning studies are made at Beltsville, Md.; at St. Paul, Minn., in cooperation with the Minnesota Agricultural Experiment Station; and at Davis, Calif., in cooperation with the California Agricultural Experiment Station.

F. Fallout protection work for the farmstead is conducted at Beltsville, Md., and selected field locations. Liaison is maintained with the Office of Civil Defense, DOD, and other appropriate agencies.

The Federal effort in this Research Area totals 7.7 professional man-years. Of this number 0.1 is devoted to meteorological factors; 0.1 to standards for serviceability, safety, etc.; 1.9 to materials and construction methods; 2.0 to water supply and wastes disposal; 1.2 to farmstead planning; 1.9 to fallout protection; and 0.5 to program leadership.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Meteorological Factors

Preliminary maps showing distribution of snow load probabilities for different climatic regions of the United States were completed during the year by the Weather Bureau under a contract arrangement. These maps are based on 10 years of Weather Bureau records on water equivalent of snow pack at their first order weather stations.

B. Construction Standards

At Beltsville, Md., analysis of the distribution of snow load probability data from the Weather Bureau maps indicated that they do not fit the mathematical model well at the 25 and 50 year recurrence intervals. Redistribution of the data will be made and maps prepared for use as loading standards for design of farm buildings.

C. Materials and Construction Methods

1. Stressed-skin panels. At Blacksburg, Va., continued research into new methods of utilization of materials resulted in improved shear connections in insulated panels faced with 1/2-inch thick portland cement mortar, reinforced with prestretched steel wire. Panels were developed that are 37 percent stronger in bending than those previously built. Panels were cast 32' long and later sawed to length. Temperature differential of 50° across the panels did not damage them. Commercial production of the panels is feasible.

2. Hyperbolic paraboloid (HP) shapes for farm structures. At Beltsville, Md., testing of various elastic materials shaped into hyperbolic paraboloids has shown conclusively that the pure membrane theory advanced for design of concrete HP's is inadequate. Secondary stresses have been determined to be more important than the membrane stress. Bending of edge members and distribution of the configuration of an HP toward a new shape--compressive arch for materials like concrete, and tensile catenary for materials like plywood--that naturally utilize the capability of the material, dictates development of more sophisticated and more accurate design formula for these shapes.

Also at Beltsville, plywood covered with a thermo-setting elastomer was used in the erection of a pentagonal building of hyperbolic paraboloids. The new shape used for investigative probing into possibilities of better space arrangement of structural members and in use of non-structural coverings for structural strength is one of two special designs in use of HP's. The other, in the form of a 1/4 scale model, employed identical HP's for wall and roof units. Currently tests are being made on practicability of fabricating quadrants of a unit in approximate 8' x 8' dimensions so the quadrants can be fastened together.

3. Rotational strength of nailed joints. Research at Blacksburg, Va., on resistance of nailed joints to rotation, showed a correlation of $r = 0.91$ in the linear regression equation of:

$$M/J_m = 44.51 + 24559 \theta$$

M = Moment on a nailed joint, inch pounds

J_m = Joint modulus, computed as the maximum lateral resistance of a nail times the ratio of the summation of the square of nail distances to the center of rotation of the joint divided by the distance to the farthest nail from the center.

$$J_m = F_{\max.} \frac{\sum r_i^2}{r_{\max.}}$$

θ = Joint rotation in radians

This equation held for 108 tests made on lap nailed joints in 2 x 4's, 2 x 6's, and 2 x 8's and thus may be used to predict strength of nailed joints.

4. Rigid timber frames. At Blacksburg, Va., a series of full scale tests on knee braced rigid frames with trussed overhangs on each side proved that assumptions made in analytical design of the system were valid, but the experimental design was not rigorous enough to extrapolate the results to prove validity of assumptions for other similar designs.

5. Air flow resistance of curtain wall materials. At State College, Miss., a study of the air flow resistance of curtain wall materials used on broiler houses gave basic data for computation of air movement through curtain walls. Limited experiments with broiler production in houses with air-tight curtain walls versus those with air-permeable curtain walls indicated that some degree of air permeability is desirable.

6. Grain bin bolt washer exposure tests. At the request of ASCS, high temperature, soak and bake, freeze-bake, and ozone exposure tests have been made at Blacksburg, Va., on washers to go under the heads of grain bin bolts. Final comparative criteria tests between the test washers and conventional neoprene washers will include tests for leaks under pressure in a specially constructed pressure chamber.

7. Plan development. A plan for a 40-foot truss for use in pole construction was drawn at Beltsville, Md., for inclusion in the Cooperative Farm Building Plan Exchange. The design was developed and field tested by the Oregon State University. Plans developed for crop structures, farmhouses, and livestock structures are reported in Areas 7, 8, and 9, respectively.

D. Water Supply and Wastes Disposal

1. Farmstead water demands. Studies on farmstead water demand and requirements are continuing in Maryland, in cooperation with the Agricultural Experiment Station.

Development and trial use of a digital automatic water use recorder for obtaining data on the complex water use involved in the operation of the present-day dairy farmstead is nearing completion. This equipment is being developed as a research instrument which will automatically record the time, amount and rate of water use at numerous points on the farmstead. In the process of developing this equipment, water use data are being collected on four dairy farms in the State of Maryland. These farms have excellent production records, good physical plants and good management, so the data obtained are expected to present water use information which will point up the requirements of good dairy farms. The data are being analyzed in a data reduction process and, through the use of analytical models, the results will be presented as basic water use and design requirements for dairy farmstead water systems. Work is also being conducted to determine the analytical model which will provide the optimum design basis. It is anticipated that in using such an analysis, the water system will not be designed to meet the extreme case but will provide the optimum adequate water system for the requirements of the particular farmstead.

2. Farmstead manure disposal. Studies are underway at College Park, Md., in cooperation with the Maryland Agricultural Experiment Station to determine design requirements for manure disposal lagoons. In an effort to establish the first basic design requirement, research equipment and techniques are being developed to determine the Biochemical Oxygen Demand (BOD) requirements of the various animal and poultry manures. Initial tests have shown that cow manure from animals fed silage only has a 5-day BOD of 1/2 pound of oxygen per pound of dry weight. It is expected that it will be necessary to determine the BOD requirements of manure from all farm animals as well as the effect of all the components of various feed programs now being used.

Laboratory work is also underway to determine the relative soil sealing effects of the various farm animal manures when added to disposal lagoons. Initial work has been with the Manor soils found in Howard County, Maryland. Using a loading rate of .01 pound (dry weight) of manure per day per cubic foot of water in a soil bin with standardized porosity, it was found that different types of manure sealed the soil in different periods of time. The respective sealing times found for the various manures were: Cattle, 20 days; hogs, 39 days; chickens, 59 days (averages of 8 trials each). These values are for the purpose of showing relative sealing effects only and are not directly applicable to undisturbed soils.

Work was done to determine the rate of sludge buildup at the above rate of loading. This buildup, which has an important bearing on the size and operation of a lagoon, was found to be as follows for a one-month period of manure loading in the laboratory: Cattle, trace; hogs, 1 mm; chickens, 3 mm.

E. Farmstead Planning

1. Chore time standards. At St. Paul, Minn., basic work in cooperation with the Minnesota Agricultural Experiment Station on establishing time standards for farmstead operations has been concerned with a search for a suitable standard for coordinating data taken under differing conditions, as well as the establishment of the actual standard data. Thus far the search for a suitable basic standard for coordinating data has been unsuccessful. In lieu of such a standard, Industrial Engineering standards are being used. The researcher's ability to rate the performance is being utilized for the elements not suited to those standards. The standards established thus far have been for the handling of baled hay, herringbone milking room operations and tractor and trailer operations. To be most effective and useful, standard times will have to be established for all farm work elements and be set up into a complete schedule. The work on this project is continuing in that direction.

2. Farmstead model layout studies. Also at St. Paul, work has continued on the use of models for analyzing the effectiveness of farmstead layouts. Previous work with models has shown that they present an excellent rapid visual picture but not a factual basis for selecting one layout out of a possible three or four that might appear to be of equal value. The final selection of the best layout is dependent upon some other factual method of selection still to be determined. The supply and variety of models completed thus far appear to be satisfactory for analyzing present concepts of dairy farmstead operations. For new and different ideas, new models will have to be developed. Initial attempts to analyze other types of livestock enterprises indicate a need for an almost completely new supply of models. Use of the models has shown a plastic film imprinted with a suitable scale grid and overlaid with a 1/4" Plexiglas sheet to be the most satisfactory base for study purposes.

3. Expansion of urban developments into agricultural areas. At Davis, Calif., in cooperation with the California Station, information on rural code and zoning problems generated by the continued expansion of urban populations into formerly agricultural areas was developed and submitted to the Western Regional Farm Building Plan Exchange Committee.

4. Layouts for handling wafers on large farmsteads. Also at Davis, preliminary, exploratory studies on efficient arrangements for transportation, storage and feeding of hay wafers on large California farmsteads have been started.

F. Fallout Protection

At Beltsville, Md., the new man-made threat to agricultural productivity, radioactive isotopes, necessitated investigation into means for protection of farm animals and their caretakers from gamma rays. At the request of FES and in cooperation with OCD, protective structure designs were studied, two plans were developed, and manuscript material for a bulletin on fallout protection was prepared. The two plans illustrate concepts of: (1) a family shelter adjacent to the livestock, (2) a multi-use shelter to offset some shelter expense by daily production, (3) a shelter that is used daily so the operation within the shelter will be familiar routine, (4) a shelter with permanent shielding, and (5) emergency standby equipment for self-sufficient operation.

PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

A. Meteorological Factors

None

B. Construction Standards

Teter, N. C. 1962. Report of construction standards committee. Presented at Summer Meeting, ASAE, Washington, D. C., June.

Teter, N. C. 1962. Minimum structural standards for rural housing. Presented at Western Region Meeting of the Plan Exchange, Jackson, Wyoming, July.

Yeck, R. G. 1962. Codes and standards for fire safety in farm structures. Presented at Farm Fire Safety Seminar, Huntley, Illinois, September 27.

C. Materials and Construction Methods

Liu, R. C., Teter, N. C., and Kent, T. E. 1962. Use and performance of sheet strips warped to approximate a hyperbolic paraboloid roof. Transactions of the ASAE, V. 5, No. 1, pp. 68-74.

Kent, T. E., Liu, R. C., Teter, N. C., and Meador, N. F. 1962. Grout surfaced, insulated, stress-skin panels for farm buildings. Transactions of the ASAE, V. 5, No. 2, pp. 165-167, 171.

Kent, T. E., Teter, N. C., and Liu, R. C. 1962. Farm building panels. USDA, ARS 42-65, August.

Three utility trusses--lap nailed construction. 1962. (Exchange Plan Nos. 5921, 5922, and 5923). USDA Misc. Pub. No. 909, August.

Kent, T. E. 1962. Broiler housing - an argument for better construction. Proc. of 1962 Broiler Housing Seminar, University of Delaware, Georgetown, Delaware, September.

Teter, N. C. and Ota, H. 1962. Insulating materials and vapor barriers--their value and application. Proc. of 1962 Broiler Housing Seminar, University of Delaware, Georgetown, Delaware, September.

Christmas ornament inspires design for new farm buildings. 1962. USDA, Agricultural Research, page 14, December.

Kent, T. E., and Dowdy, P. L. 1963. Use clear-span roof trusses. Hoard's Dairyman, pp. 210-211, February 25.

D. Water Supply and Wastes Disposal

Eby, H. J. 1962. Manure disposal lagoons. Presented at Symposium on Industrial Waste Control, Washington College, Chestertown, Maryland, May 5.

Jones, E. E. 1962. Measuring water use. Water Well Journal, August.

Eby, H. J. 1962. Design criteria and management for manure lagoons. Agricultural Engineering Journal, December.

Rockey, J. W. 1963. Water requirements and treatment for the home and farmstead. Agricultural Engineering Journal, January.

E. Farmstead Planning

Larson, R. E. 1962. Traffic patterns of animals, people, vehicles, feed and materials. Presented at Farmstead Planning and Mechanization Workshop, University of Illinois, Urbana, Illinois, December 4.

Cleaver, Thayer and Kelly, C. F. 1962. Drylot feeding facility design for California. Presented at Winter Meeting, ASAE, Chicago, Illinois, December 11-14.

F. Fallout Protection

See publication, Area 7C.

CROSS COMMODITY AGRICULTURAL ENGINEERING RESEARCH
Agricultural Engineering Research Division, ARS

Problem. The best placement for starter fertilizer is unknown for a number of crops in different areas of the country. There is a need for development and testing of fertilizer application equipment for unusual crop situations, such as hillside orchards, sugarcane, tree transplants, etc. There is need for considerable fundamental study of small particle behavior, of radically new methods of applying chemicals, and of the movement of liquid and gaseous chemicals in the soil.

There is need to develop engineering design criteria for constructing and equipping plant growth chambers that will reliably provide and maintain desired thermal, lighting, and other environments over a wide range of experimental conditions. While research on harvesting equipment and methods has led to much improvement in the reduction of production costs of such crops as grains and forage, much additional work needs to be undertaken, both basic and developmental, in order that all crops may be mechanically handled. Better methods, techniques, and equipment are needed for use on farms for the initial preparation for market or the processing of farm products to increase efficiency in the use of labor and equipment, preserve quality and prevent spoilage and damage from mechanical handling.

Further research is needed on devices for substituting electric energy for hand labor for many farmstead operations. Some electrical treatments have been found to accelerate germination and seedling emergence. If emergence in the field can be speeded up and better uniformity obtained, weed control can be much more effective, with resulting increased efficiency in production of crops. Treatments also increase the percentage of germination for some seeds and would therefore enable the establishment of good stands with lower investments for seed. Increased use has forced many farmers to rewire or partially rewire their farmsteads at considerable cost. Overloading of installed wiring results in poor equipment performance, energy losses in the wiring, and creates a fire hazard. Transformers burn out or must be replaced due to overloading. There has been no good method of predicting when a transformer should be replaced.

USDA PROGRAM

The Department has a continuing long-term program involving agricultural and other engineers, architects, physicists, soil scientists, and statisticians engaged in research to apply engineering principles to agricultural production.

Studies of means of applying fertilizer most effectively are in progress at 26 locations in 13 states. At Wooster, Ohio, basic research is conducted on fundamental studies of aerosols and on various spray formation devices.

Typical plans for crop structures and related equipment are developed at Beltsville in cooperation with the regional committees representing all State Experiment Stations and Extension Services. Long fiber crops harvesting research at Belle Glade, Florida, is cooperative with the Everglades State Branch Experiment Station. The decorticating, retting, and cleaning of long fiber crops is also carried on at Belle Glade in cooperation with the Everglades Branch Experiment Station, the Office of Defense Mobilization, and industrial fiber users.

Studies of ways to improve or develop new apiary equipment are underway at Tucson, Arizona, and Madison, Wisconsin. A program to develop an improved method of estimating the maximum electrical demands of farms is in cooperation with the Iowa Experiment Station, the Rural Electrification Administration, and power suppliers in Iowa, Montana, Minnesota, North Dakota, Wisconsin, Kentucky, and Alabama.

A new program at Beltsville has been established whereby engineers from the Agricultural Engineering Division cooperate with the Crops Division on basic studies of light and thermal environment and their relation to plants in growth chambers. Research on electromagnetic energy for control of insects in stored grains and seeds is carried on in Nebraska and for conditioning seed to improve germination and emergence in Nebraska, Tennessee, and Washington.

The Federal scientific effort devoted to research in this area totals 13.2 professional man years. Of this number 1.1 is devoted to fertilizer placement and distribution equipment; 0.2 to seed planting equipment; 0.3 to transplanting and fertilizing equipment; 1.0 to basic studies in aerosols and spray formation; 2.2 to plant environment equipment; 2.0 to long vegetable fiber crops; 2.0 to apiary equipment; 1.2 to energy distribution and farm electric demand; 0.7 to glow discharge radiation; 0.5 to insect survey traps; and 2.0 to decontamination of agricultural lands.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Fertilizer Placement and Distribution Equipment.

Forty-eight experiments on the establishment of field crops and vegetables were put in cooperatively with various State, Federal and commercial research units. Thirty-two experiments were handled out of the Beltsville Station, three by the Southeastern Station, and thirteen

by the Southwestern Station. Some typical results of the cooperative field experiments are: A new technique of measuring or detecting action of fertilizer-seed placement was devised by this Investigation Unit the past season to complement field experiments with cooperating plant scientists. A special field machine was designed whereby the seed and fertilizer's action with the soil may be closely observed and tied in with unearthed seed-fertilizer position. This method employed high speed motion photography on a field testing device so that soil performance tests could be observed by an eight to one reduction of time (or speed - by slow-motion film). This study was made as a part of a planting-fertilizing study with small grains. In 1962, these field experiments on small grains were continued with Michigan and Indiana (winter wheat, spring oats) and separation of seed and fertilizer showed benefits on high rates of fertilizer application (up to 15 percent greater production). Several companies are advertising separate seed-fertilizer openers for drills, but many plant scientists feel the type of separation, if any, is not desirable for efficient use of the fertilizer. In a series of these tests on commercial seed-fertilizer openers with high speed photography only one of four openers tested in field performance trials gave separation of seed and fertilizer - and the placement of this one opener was not satisfactory in the opinion of the cooperating plant scientist.

From these trials, the most promising openers were studied in great detail, with the objective of designing an opener that would have good field performance. A successful 1 X 1 inch side placement opener with a seed firming wheel was made which, in turn, was used on seven experiments in 1962 with good performance; application for patent of the new opener was made; and one company has made a new opener almost identical to this development and it has been sent to several State Agricultural Experiment Stations - in April, 1963. Other companies have discussed the trials reported at the National Meeting and apparently are planning on changes on their present openers.

B. Basic Studies in Aerosols and Spray Formation.

The objective of the research conducted at the Pioneering Research Laboratory on Physics of Fine Particles at Wooster, Ohio, is to obtain basic theoretical and experimental information as a contribution to fine particle physics which should ultimately result in improvement of efficiency and distribution characteristics of pesticide application. Theoretical work included the following: (a) development of a mathematical treatment, based upon conditional probability theory, for Brownian motion systems where particles are of non-uniform size; (b) how to represent initial distributions of soil fumigant mathematically in, and the solution of, some typical problems on the diffusion of fumigant in the soil; (c) the solution of a particular stochastic (statistics with a time parameter)

differential equation; (d) a treatment of measurements and the mechanics of droplet impaction based on stochastic theory. Stochastic analysis of the distribution of aerosol deposits was also studied, as were theoretical models of turbulent diffusion in aerosol mechanics. Experimental work involved the development of an ultraviolet exposure unit for spectrographic study of fluorescent tracers for aerosol deposit measurements. Data for several tracers and other fluorescent materials have been obtained and are being analyzed. Special methods for spectrogram density recording were applied to study of the fluorescent spectra.

Studies of thermal and moisture diffusion in pastes of fine starch particles are being made in cooperation with the USDA Soft Wheat Quality Laboratory, Wooster, Ohio. Studies of digital computer methods for solving water gravity-flow problems in soils were conducted in cooperation with the Ohio Station.

C. Plant Growth Structures

1. Environmental chambers. At Beltsville, Md., studies to date have been concerned principally with improving existing facilities. Equipment and instrumentation for measuring and recording data on the various thermal factors (temperatures, air flows, relative humidities, etc.) involved are being acquired or developed.

Several small chambers, needed for plant physiology research, were used experimentally to test means of maintaining uniform ambient temperatures through a range of levels of light intensity. The chambers were inside an air-conditioned room. Small exhaust fans were installed in the chambers and set to blend the warmer chamber air with incoming cool room air at the room ceiling. The installations are operating successfully and a report of design details is being prepared.

A means for protecting the temperature controls of a "hot room" for treating virus infected plants was developed and tested and found to perform satisfactorily. Initially the controls had been installed inside the hot room where the high temperature and humidity caused rapid deterioration. Re-design of the system and placement of the controls in an electrically warmed, insulated box that provided a uniform temperature solved the problem.

Several experimental modifications were made on a commercial growth chamber at Beltsville, Md., during the past year. The growing area was divided lengthwise to allow testing of two types of lamps. Recording instruments were placed on the chamber to measure compressor pressure, temperature, and humidity of the growing area, intensity, quality, and operating time of the fluorescent lamps, and air velocity. Continuous testing of plant responses to light quality has been carried on since

July 1962. All tests compared "special phosphor" fluorescent lamps to "cool white" fluorescent lamps. Some also had incandescent light supplementing each quality fluorescent light. Pinto beans were slightly heavier under "cool white" light than under "special phosphor", both with and without incandescent light. Incandescent light caused the beans to elongate more, but made no difference in weight. Beans under "cool white" light were longer than those under "special phosphor" without incandescent light, but were shorter when "special phosphor" was supplemented with incandescent light. Marigolds produced more blooms under "cool white" light in the same test. No appreciable difference was found with oats and African violets. All plants under both sources of light grew well. Problems of overheating of lamp ballasts during the hot summer and condensation of refrigerant during the coldest winter nights were studied and successful corrections developed.

Several monitoring instruments have been developed and successfully tested for obtaining a record of conditions within environmental chambers of different types in different types of operations. Monitored conditions included humidity, temperature, air flow, refrigeration equipment operation, and other quantities. The information obtained will serve as design criteria for these chambers.

Initial studies at Beltsville have also been concerned improving existing facilities, equipment and controls associated with plant growth chambers. Instrumentation to measure and record performance characteristics of both components and systems is being acquired. Such measurements include temperature, relative humidity, air flow, barometric pressure, light and radiant energy.

Special lamps and light sources for producing far-red radiation were tested for performance. Commercially available lamps require optical filters and special cooling. Efforts are in progress to find satisfactory methods of using these commercial lamps without the losses in desirable radiation accompanying conventional methods of cooling and filtering radiation. Fluorescent type light sources specifically designed for plant growth applications are being compared with standard fluorescent lamps and incandescent lamps in respect to both plant response and lamp performance.

Lamp performance and associated components have been troubled by early failure, improper installation, and rapid deterioration. Factors involved include supply voltage, ambient temperature and ventilation, lack of protective safety controls, inadequate design of lamp and associated components, improper installation and lack of monitor records of performance.

Controls for both lamps and heating-cooling apparatus are being replaced, modified, rearranged or increased to give both performance and protective type action. Monitor information in existing chambers is being secured to

establish basis of performance required by plants of growth chamber apparatus. For example, Dunmore type humidity apparatus is being utilized to secure continuous relative humidity levels in growth chambers. Data on permissible relative humidity levels desirable in growth chambers exists only for very limited specific environmental situations.

Light and radiation measurement equipment of laboratory type is being assembled to determine absolute spectral output of various light sources before, during, and after use in growth chambers.

In cooperation with industry, a spectroradiometer is being built for primary use with plant growth chamber lamps and related agricultural applications.

2. Greenhouses. At Beltsville, Md., a temperature-humidity recorder was placed in an air-conditioned potato greenhouse to obtain information that could be applied to air conditioning of other greenhouses. By comparing the data obtained from the air-conditioned potato greenhouse and from an existing sugar cane greenhouse, it was determined that humidifying equipment was not necessary for normal sugar cane growth in an air-conditioned greenhouse.

Models of three small, low-cost, plastic covered greenhouses for home gardeners were developed, tested, and evaluated. The final design is economical, easily built, strong, and durable.

In cooperation with the Washington State University, Pullman, Washington, equipment and facilities for measuring, recording, and controlling the carbon dioxide concentration is in operation in three greenhouses. The system has been tested for one complete winter and has proved satisfactory. The distribution of CO₂ within the plastic greenhouses varied approximately \pm 3 percent from the control set-point. Growth rates of bean plants and production of tomatoes were greatly increased by carbon dioxide levels 3 times normal atmospheric concentrations. Six times normal concentrations produced slightly less increases, although in both levels there was indication of improved photosynthetic efficiency.

D. Plan Development

Four typical plans for crop storage, and plant growth structures and equipment were developed during the year at Beltsville, Md., for inclusion in the Cooperative Farm Building Plan Exchange. They are: a small (8'-6" x 12'-0" x 7'-0" high at the ridge), plastic covering over plywood frame, greenhouse for home gardeners; a 5'-4" x 7'-8" x 7'-8" high, plastic covered, wood-frame, greenhouse--coldframe; a lightweight, 36' long trailer for 30'-40' length irrigation pipe sections; and a small, dual-purpose, fruit and vegetable storage cellar that also serves as a fallout shelter for six persons.

E. Long Fiber Crops Harvesting Equipment

1. Kenaf and other jute-like fibers. About 12 tons of retted fiber produced at three locations, namely, Belle Glade, Florida; Palm Beach County Farm, Florida; and Tifton, Georgia; were shipped to two mills for experimental spinning tests. The acre yield averaged 1,250 pounds and costs were estimated at eight cents per pound. The grade of fiber compared with kenaf imported from the Orient showed a value of 10 cents per pound, the quality being in every way equal to the imported product. Improvements on the equipment included the development of a mechanical splice for the grip belts; replacement of gasoline engine drive for second ribboning drum by a hydraulic motor; and clearing passageway through the machine to prevent trash or other material fouling chains, shafts, etc., thus reducing stoppages for cleaning. Performance of the harvester-ribboner was considered very satisfactory as only minor difficulties were encountered throughout the season. The harvesting rate was .86 acres per hour. The mechanical washing equipment was rebuilt, eliminating the scutching unit and adding several pairs of squeeze rollers. The entire unit worked very well but the handling of the fiber from the harvester through retting and cleaning needs improvement. A complete harvester-ribboner and washing unit patterned after the USDA equipment have been built by a farm equipment manufacturer under contract to AID for use in Sudan. It is anticipated that the equipment will be used to harvest and process about 200 acres of kenaf.

2. Sansevieria. The objectives of the program are to develop a combine type harvester-decorticator, to determine the best method of treating the field harvested fiber to the baling stage; and to determine harvesting techniques that will result in the best regrowth of new plants from the rhizome bed. Due to major project emphasis being placed on other fiber crops, work this year has been confined to the redesigning and rebuilding of the experimental harvester-decorticator to handle a heavier volume of leaves. Conversion of the machine to a self-propelled unit was also initiated. This is needed to keep the swath of plants accurately in line with the gathering chains and the divider separating the cut leaves from those left standing. The sansevieria plantings at all locations suffered moderate to severe damage from the cold spell in December when the temperature dropped to 28° F. Regrowth of the damaged plants is under study.

F. Decortivating, Retting, and Cleaning Long Fiber Crops

During the past year studies on improving processes and techniques for cleaning ramie ribbons has been devoted to work on an in-line type degummer developed by the late Charles Short. This process degums ramie fiber from well decorticated material, and is also applicable to kenaf fiber. The original machine has been redesigned by Experiment Station personnel and

a private individual, but needs to be completely rebuilt and stainless steel chains and sprockets installed. This project has contributed material and personnel in making the machine operable for the limited tests made thus far. The process in its present state does not clean ribbons well enough for commercial use, but improvements in this machine and in the field ribbons produced appear possible. The three and one-half acre planting at the Everglades Experiment Station is being maintained as a source of material for harvesting and degumming tests.

G. Apiary Equipment

In Wisconsin the development of mechanical equipment for extracting honey and manipulation of large two-queen hives suitable for use in the North Central states is being developed in cooperation with the University of Wisconsin Agricultural Engineering Department and the Apiculture Research Branch, ENT.

A control system for an electrically heated cappings melter is being developed. A system of two thermostats which control the heaters through a relay is being used to control the temperature so that the wax is brought above the melting point without injuring the honey.

An electrically heated uncapping knife is being developed which will maintain a constant temperature level of the knife for various rates of uncapping. An electronic temperature controller will be used to establish the control requirements.

The nylon cloth strainer having 430 square inches of straining surface has been used and has proved very satisfactory. Two strainers installed in series are used. The strainers are cleaned after three hours of operation during which time approximately 3,000 pounds of honey was strained that had been uncapped with a vibrating knife. Cleaning time is less than 10 minutes. These strainers, used in a system of heaters and coolers, provide continuous flow conditioning at recommended temperature levels.

Time required to fill a 60-pound can with honey is limited only by the tank discharge tube when using a bellows-operated, automatically controlled, filling valve. Restrictions in the valve body have been removed.

Removal of bees from filled honey supers with streams of high velocity air shows promise. A shop type vacuum cleaner used as a blower with a rectangular shaped nozzle having 1/2 square inch of area was used. Further study of various fans and nozzles is planned.

A bee watering device consisting of a large flat pan with a coarse synthetic sponge 2-inches thick floating on top of the water is being used. A cover 3 feet above the sponge surface prevents water contamination from flying

bees. Water level is maintained by a float valve connected to a pressure water system; otherwise, the waterer has no moving parts. A sponge with a coarse cell structure is more satisfactory than one with a small cell structure.

Testing of plastic combs having various cell shapes and made from different materials to determine their acceptance and use by bees for brood rearing and honey storage is being done to explore the possibility of material to be used in hive construction. Round, hexagonal, and square shaped cells have been used thus far. All have been accepted and used by the bees, the round cells being most acceptable. Honey stored in the cells could not be uncapped with a knife due to concave cappings below the cell edge.

In Arizona the development of mechanical equipment suitable for apiary operations in the Southwest is being developed in cooperation with the University of Arizona Agricultural Engineering Department and the Apiculture Research Branch, ENT.

Plastic comb made of polyethylene with 3/16-inch round and 1/4-inch square holes were tested at Tucson and Madison, Wisconsin. Some of these combs were coated with beeswax before placing them in the hive. The results were not conclusive but showed promise enough to further the test with 10 different thermoplastics. These will be tested in 1963 at both locations. Three hole sizes will be used. The object of this work is to find a material that is suitable to the bees and will be a uniform and rigid comb for automatic uncapping.

The patent for the pinned uncapper roller was received and a new design of the roller was made. This machine is being designed to increase the efficiency of operation of the portable extractor.

Painting of hive bodies is a major maintenance problem. Seven paints in a total of 22 combinations were used on hive bodies for a durability test. In 8 months the aluminum paint has failed. Temperature measurements just under the paint showed aluminum the warmest with no difference between white paints.

Studies were made to determine how air circulated in the hive, how air affected the hive temperature and how radiation affected the hive temperature to find better means of protecting a colony from heat and insecticidal damage. The studies were performed under controlled condition where one variable was changed per test. Either cold or hot air blown into the hive changed the temperature at the place of entry quickly but in the rest of the hive it was slow in changing. The rate of circulation in the hive depended somewhat on the strength of the colony and on the hive configuration used in the test. Thermal radiation had very little effect on the hive temperature except areas adjacent to hive wall at place of application. The conclusion was that the circulation of air within the hive is very limited.

Cooperative work with the University of Arizona Entomology Department was performed on shades for the leaf cutter bee. Several shade materials and nesting materials were tested, but due to an infestation of the chalcid fly, the test was terminated without conclusive data that any one material for shade or nest was better than the others. Presently they are making a controlled temperature study on the life cycle of the leaf cutter bee which has value in pollination.

H. Energy Distribution and Farm Electric Demand

In cooperative work with the Iowa Agricultural and Home Economics Experiment Station, power suppliers, the Rural Electrification Administration, and members of the Farm Wiring Committee of the American Society of Agricultural Engineers, a method of estimating the required transformer sizes for individual farms is under development. This method makes use of multiple-regression equations in estimating the maximum demands of consumers. Demand predictors are energy consumption and the major appliances owned. Coefficients for multiple-regression equations were calculated from data collected for this purpose on 714 farms in 8 northern and central states. These equations and a related transformer-sizing procedure are now being field tested by three power suppliers. Analysis of data from Alabama shows that the maximum demands of farms in this state cannot be accurately estimated by the equations developed for Northern and Central United States. Coefficients for two equations were calculated from the Alabama data, one for farms without and one for farms with electric house heating. Satisfactory estimations of the maximum demands of Alabama farms can be made by use of these equations.

The demands and connected loads of the buildings on 103 farms were tabulated as part of a national farmstead wiring study. The objective of the study is to determine factors which may be applied to the connected loads of farm buildings in sizing service entrance equipment. The demands of several hundred additional farms are now being metered. The incomplete information indicates that a demand factor of approximately 0.5 may be applied to connected loads in excess of 10 kw. Approval and publication by the National Electrical Code of the findings of this study will result in reduced wiring costs for many farms without increasing the risk of fire.

Further work includes the completion of the study of required service-entrance capacities of farm buildings, the development and test of transformer sizing procedures with special attention to farms in the South and to consumers with low energy consumptions, and an analysis of voltages and wiring methods for distributing electricity on farmsteads.

The addition of one silo unloader and a bunk feeder adds nearly ten horsepower to the connected load of a farmstead wiring system. Many new installations add two or three times this amount. The use of 480 volts to operate these additions seems worth considering. Our explorations in this area have brought to light many new complications. Many state codes (Minnesota included) limit voltages to ground on single-phase services at 150. This rules out 440-volt, single-phase motors. In addition, single-phase motors rated for this voltage would have to be a specialty item, at least at the beginning.

The same state codes limit three-phase industrial service voltages to ground at 300. This would dictate a Wye connection of the three transformers. The distribution line serving the Agricultural Engineering farmstead is a Delta system with a midpoint ground on one transformer. The only way to meet the voltage limitation to ground would be to operate with a phantom center ground. This is not compatible with the other service connections on the line.

The use of 480 volts for distribution and a step-down to 240 volts at the silo are now being investigated. This possibly would be allowed by the Minnesota code.

A mechanical problem has arisen in connection with grounding a three-phase motor on a silo unloader. All standard transition devices come with three slip rings. What few three-phase motors have been sold have been grounded through the metal housing part of the transition piece. Safety specialists would prefer an extra slip ring or a ball bearing connection in the transition.

I. Basic Characteristics of the Glow-Discharge Radiation

Studies were continued at Knoxville, Tennessee, on voltage and current wave forms associated with glow-discharge treatment of seeds. The significance of differences in wave forms noted under different operating conditions has not yet been satisfactorily explained.

Temperature studies in the glow-discharge apparatus have continued at Pullman, Washington, with the aid of encapsulated chemical compounds of known melting points. Information on the temperatures in the discharge tube indicates that, for a given treatment condition, a particular temperature is reached sooner with seeds in the tube than with no seed present. Temperature measurements in the discharge tube will be continued and a study of the relationship between these temperatures and the effects of the treatments will be made.

7. Decontamination of Agricultural Land.

1. The effectiveness of various common types of farm and industrial machinery for removing fallout from farmland has been determined from investigations made during the past four years. The percentage of removal to be expected with the various types of equipment is now known, however, other removal factors such as the disposal of the contaminated soil, are still a problem. During 1962 Tayland winter wheat was grown to maturity on two types of soil for use in fallout tests. After contamination the wheat was removed with a combine, the stubble-covered ground was pulverized with a disc, and was decontaminated with a flail forage harvester or a street vacuum sweeper. Due to a poor isotope in the fallout simulant the tests were not as accurate as previous ones; however, they were accurate enough to demonstrate that while pulverizing allowed greater removal, particularly from the heavy loam soil, the decontamination still was inadequate with suction-type equipment unless followed by a scraping operation.

In the fall of 1962 tests were run with cement slurries and commercial liquid mulch solutions on the surface soil to develop a means of localizing the fallout in inaccessible or hard to remove places to hold it in place until it becomes less radioactive. The commercial mulches were considered too expensive. Sand and cement mixed with the surface soil and sprinkled with water did not form a cohesive crust. A slurry of 3-parts sand to 1-part Portland cement and about 1-part water was found to be the most satisfactory. The slurry was applied with a modified lime spreader and allowed to set. Some of the thin crust was crushed with a disc harrow or a corrugated roller in order to make it easier to remove. Attempts were made to remove both the crushed and solid crust with a rotary broom street sweeper and a side delivery rake. The rake proved satisfactory on both the broken and unbroken crust on the sandy soil not covered with vegetation.

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PIONEERING PLANT PHYSIOLOGY AND VIROLOGY
Crops Research Division, ARS

Problem. Producing adequate food supplies is largely dependent upon continually developing improved crops of high quality, resistant to diseases and adverse environmental conditions, controlling plant diseases and weeds, and developing improved cultural methods. Current progress is based on the application of basic principles learned in the past in physiology, plant virology, and other sciences. To insure continued progress, basic research in biological sciences must be increased to solve many of the problems that now limit further significant increases in yields and in developing higher quality crops, to learn how to control plant diseases, and to improve present methods of altering plant growth and development by environmental means such as light and chemically by use of growth regulators. The mechanism of action of the plant pigment in plants responsible for controlling growth and development is unsolved. We are not fully utilizing the potentialities of regulating chemicals to solve agricultural problems. We need to know how growth retardants affect the behavior of plants so that we can effectively use these compounds to increase the resistance of plants to extremes of temperature, to drought, and to high soil salt content. Basic information is needed concerning the absorption and translocation of regulators and other organic chemicals so that more effective plant regulators and systemic pesticides can be developed. New, more effective safe chemicals are needed for plant disease control. Currently there is no satisfactory chemical control for viruses or soil-borne diseases. Viruses are one of the major classes of diseases reducing crop yields. Yet little is known of their structure and behavior in plants and soils. With more emphasis on the basic biological sciences, new principles that may be discovered will enlarge the possibility of our ability to maintain and perhaps increase the production of agricultural crops.

PROGRAM

The Crops Research Division devotes approximately 38 percent of its professional manpower to basic research. Only a small segment of research in plant physiology and plant virology, that of the three Pioneering Laboratories, is reported here.

Plant physiology

At Beltsville, Md., a small group of scientists are exploring more deeply into the effect of light in controlling plant responses such as seed germination, stem elongation, flowering, and pigmentation.

The reaction of many plants to various periods of exposure to light of different quality is known but additional plants thought to be unaffected by photoperiods are being restudied in view of recent basic discoveries of the light response in plants. Biochemical research is under way to purify the pigment, phytochrome, now known to control the growth and development of plants.

New compounds from universities, chemical companies, and government laboratories are evaluated as regulators. Their effects on plant growth and development are studied. Responses that represent improved methods of crop production are tested cooperatively with crop specialists. Agricultural sources of plant regulators are being sought and attempts made to isolate these hormones from plants. The fate of regulating compounds and physiological mechanisms involved when plants respond to these substances are being studied. Through cooperation with chemists at the universities and in industry, growth regulators with different molecular configurations are synthesized for this purpose and studied to learn how their movement in plants can be affected. Effects on crops after harvest, such as ripening, retention of vitamins, etc., are studied in cooperation with Agricultural Marketing Service.

The Federal scientific effort devoted to research in this area totals 10 professional man-years. Of this number 5.9 work is on photoperiodism and 3.9 on growth regulators and 0.2 to program leadership. A 5-year P.L. 480 contract for \$56,754 equivalents in Indian rupees with Calcutta University provides for basic research to isolate growth regulators from plants. In Israel, the effect of drought on translocation and metabolism of organic substances in plants is being studied under a 3-year P.L. 480 contract for \$35,177 equivalents in Israeli pounds with University of Jerusalem.

The occurrence of natural growth regulators in citrus under different environmental conditions is being investigated in Israel under a 5-year contract for \$56,754 equivalents in Israeli pounds with University of Jerusalem. A 5-year study of the response of rice and onions to various light durations has been initiated with the University of the Philippines under a contract for \$20,920 equivalents in Philippine pesos.

Plant virology

In the Plant Virology Laboratory, Beltsville, Md., studies are conducted to determine the physical and chemical nature of viruses the manner by which they enter, move, and multiply in plants, the process of inducing a disease response in plants, and means of

identification and classification through serological techniques. Special emphasis is on insect-transmitted viruses such as aster yellows because this group has been difficult to purify and study by physical and chemical means.

The Federal scientific effort devoted to research in this area totals 4.0 professional man-years. Of this number 3.9 is on plant viruses and 0.1 to program leadership.

PROGRESS

A. Plant Physiology

1. The Nature of Photoperiodism.

a. Flowering in *Chenopodium rubrum*. Further studies of photoperiodic control of flowering in seedlings of *Chenopodium rubrum* revealed important facts about the way phytochrome acts. Far-red light given for a few minutes in the middle of each long night immediately after a few minutes of red light was found to counteract the flower-inhibiting effects of the red. Such reversibility was not encountered in all short-day plants investigated, but was evident in many. However, if the far-red light was applied in the middle of the night for an hour or more, whether or not preceded by red, flowering was markedly reduced and often completely inhibited in a number of short-day species. The explanation for these opposite effects of brief and prolonged treatments with far-red on flowering were examined in detail and explained on the basis of phytochrome action.

It was known that phytochrome, the active pigment for regulating of flowering occurs in two forms, one having an absorption maximum in the red at 660 mμ and the other in the far red at 730 mμ. Both forms, however, absorb weakly throughout the visible and far-red spectra. Therefore, 730-mμ radiation, which converts most of the phytochrome to P₆₆₀, nevertheless, maintains a small fraction in the active P₇₃₀ form so long as an adequate intensity of far-red light is continued. The question at issue was whether such a small amount of P₇₃₀ acting over a period of time could account for the inhibitory action of far-red light on flowering, or was the action of some photoreceptor other than phytochrome involved.

The rate at which phytochrome stops the flowering reaction in seedlings of *Chenopodium rubrum* was measured by converting phytochrome to the active form by red light, allowing it to act for measured periods and then converting it back to the inactive form by a few minutes of far red and observing the resulting level of flowering. It was found that flowering was not fully inhibited until P₇₃₀, the active form of phytochrome, had acted about an hour.

When far-red light was used continuously for various periods, the decrease in flowering response was at the same rate as when red light was used for equal periods, showing that the low level of P_{730} maintained by far red was adequate to inhibit flowering as quickly as the much higher amount maintained by red light. Far-red light maintains about 2 percent of phytochrome as P_{730} , whereas red light maintains nearly 100 percent in that form. The identical rates of floral inhibition show that between these two levels the amount of P_{730} is not the rate-limiting condition. When seedlings are irradiated with far-red light for 30 minutes, an effective level of P_{730} is maintained during the 30 minutes and for a period after the far-red light is extinguished, during which the level of P_{730} declines in darkness from its 2-percent level to an ineffective one. This latter period was found to be less than 2 minutes. Thus, far-red light applied after red reduces P_{730} from a high level to about 2 percent and the latter quickly sinks to an ineffective level in darkness and thereby stops the action of P_{730} and results in reversal of response. The opposite action of far red occurs only when the 2-percent level is not permitted to decline, which happens during periods of prolonged application of far red.

b. Rate of maturity in sorghum. Further studies of sorghum varieties that differ in relative dates of field maturity revealed no measurable differences in their phytochrome characteristics. Phytochrome clearly controls flowering of these genetically different strains, but the differences in maturity exhibited by them appear to depend on rate-limited reactions with which phytochrome is perhaps only indirectly involved.

2. Purification of Phytochrome

a. Extraction procedures. Phytochrome is extremely labile and short lived on removal from the plant. Its rapid deterioration necessitates development of new, rapid, and efficient procedures for successful purification. One such development is a new calcium phosphate chromatographic procedure. A further development is a method of preparing very small particles of agar and other gels, the small size of which markedly increases their protein-separating efficiency.

b. Physical properties of phytochrome. The action spectrum for the conversion of phytochrome in vitro has been measured from 350 to 800 $m\mu$. Two additional absorbance bands of the pigment have been determined in the blue portion of the spectrum. The conversion of the far-red-absorbing form of the pigment to the red-absorbing form requires about four times the light energy needed for the reverse conversion.

These measurements explain the different energy requirements for pigment conversion in the plant. Absorption spectra of purified phytochrome reveal an additional absorption band at 280 mμ due to the aromatic amino acids of the protein. A non-reversible form of phytochrome is now known which apparently results from partial denaturation of the protein.

The distribution of phytochrome in several dark-grown seedlings has been examined. The maximal content of phytochrome is found in the regions of potential growth and maximal protein content.

Dark reversion was studied in the cauliflower head and in a number of dark-grown grass seedlings. The results from cauliflower are somewhat different from those from the grass seedlings. This difference is not fully understood but is possibly related to the fact that no pigment is apparently lost from the P730 form in cauliflower except by dark reversion to P660. (Cooperative with Agricultural Marketing Service and Soil and Water Conservation Research Division, ARS.)

3. Plant Species Response to Photoperiodism

a. Physical and physiological evidence for the occurrence of phytochrome in dodder. Exploratory studies of dodder, Cuscuta indecora, a parasitic seed plant that lives upon a number of natural and crop-plant hosts reveal that this small non-green plant contains phytochrome. Phytochrome was detected in the living dark-grown seedlings by spectrophotometric procedures and in both light- and dark-grown plants by their physiological responses to light. The responses influenced by light include straightening of the curved hook at the end of the dark-grown seedling, and the development of the twining habit of growth by which the parasite establishes close contact with the host.

This plant, because of the low level of chlorophyll it produces and because of its parasitic habit of growth, has promise as a useful tool for further research on the physiology of phytochrome.

b. Response of Melilotus to intermittent lighting. Sweetclover, Melilotus alba, a long-day plant, flowers in winter in the greenhouse only when given artificial light at night. Flowering is earliest when light is given for several hours or continuously each night. The need for such long periods of light is apparently not to satisfy an energy requirement, however, because intermittent light applied only 10 percent of the time throughout such periods is nearly as effective as continuous light. The effectiveness of intermittent light depends on the frequency of its application and is greatest when applied in short cycles. For example, 1.5 minutes light each

15 minutes is more effective than 3 minutes each 30, or 6 minutes each 60 minutes. The effectiveness of light in 1 hour or longer cycles is actually much lower than in the shortest cycles and, if light is given in a single period equivalent to 10 percent of the night, it is almost without effect.

These results are explained on the basis of the rate of dark reversion of phytochrome. Continuous light maintains phytochrome in the active form, which is favorable to flowering. Intermittent light converts phytochrome to the active form each time the light is applied, but between light periods the active form begins to revert to the inactive state. If the intervening dark periods are short, dark reversion does not impair the flower-promoting effectiveness of the treatment. When the dark periods **exceed** an hour, however, much of the effectiveness of the light is lost.

c. Flowering responses of isogenic lines of barley. Experiments, similar in some respects to those with sorghum, were undertaken with isogenic lines of barley. These are strains of barley differing in a single gene that have been inbred many generations and are thus identical except for this single gene. The member of the pair carrying the dominant gene exhibited a typical long-day flowering response. The recessive number, however, flowered best when the plants received several short days before they were subjected to long ones. The recessive form thus appeared to exhibit the characteristics of a short-long-day plant. If this is confirmed, this plant material will be very useful for further analysis of the short-long-day type of response which has been encountered in other kinds of plants.

d. Anthocyanin synthesis in Wheatland milo. Anthocyanin synthesis and certain aspects of seedling development seem to require much higher energies than are usually associated with phytochrome responses. Studies of anthocyanin production in apple-skin tissue and milo seedlings indicate that the amount of energy required is much lower than previously supposed. The process is apparently limited by time because light given in pulses of a few seconds alternated with brief dark periods results in production of at least as much anthocyanin as does continuous light of the same total energy. When the dark period of each cycle is increased, less anthocyanin is produced. Although these studies do not prove that phytochrome is responsible for the primary photoreaction that induces anthocyanin production, the results are, nevertheless, compatible with our present knowledge of the action of phytochrome.

The first clear-cut evidence of phytochrome operating in the plant root was demonstrated in excised roots of milo seedlings. The roots produced anthocyanin which was photoreversibly controlled. As in the

shoot, phytochrome control is subsequent to a primary photo-reaction that seems to require high energies. Preliminary studies of the primary photoreaction show that cyclic lighting exerts a greater control over anthocyanin synthesis in the root than in the shoot.

4. Design of New Facilities for Photoperiodic Research

Studies are being made of the design of controlled-environment chambers for plant growth. Controlled-environment plant growth rooms are increasing both in number and complexity because almost every program of plant research from breeding to space technology requires such facilities. The demand for plant growth rooms has progressed faster than knowledge of the effects of environment on plants and has far outstripped research on proper design of such equipment. Research has been initiated on the efficiency of operation of high-intensity light sources and on the effectiveness of such sources for growing plants. A high-intensity far-red source, several plant growth cabinets, and a special-purpose lamp controller were designed and built to facilitate studies of the physiology of phytochrome. (Cooperative with Agricultural Engineering Research Division, ARS.)

5. Metabolism of Some Phthalamic Acids and the Control of Apical Dominance.

It was discovered that 3-nitro-1-naphthylenelactamide killed the terminal buds of young bean plants and induced lateral bud growth when the chemical was applied to the stems of the plants. This is the first growth-regulating property reported for this phthalamate.

This chemical is apparently degraded within the plant into another or other compounds which do not possess plant growth-regulating activity. It was, therefore, experimentally possible to apply amounts of the chemical to the stem sufficient to kill the terminal bud but within the limits of the amount the plant could rapidly degrade, thus allowing vigorous growth of the lateral buds. However, plants failed to respond to a similar application of the compound to the primary leaves instead of to the stems. Roots were apparently not affected by the chemical.

Sixty-three other phthalamic acids structurally related to the one mentioned above were studied to gain an understanding of molecular configuration and growth-regulating activity of naphthylphthalamic compounds. Fourteen of these compounds in addition to 3-nitro-1-naphthylphthalamic acid induced responses that were typical of the naphthylphthalamic family. Phthalamates with different structures were found to be degraded at different rates after they were absorbed by the plants.

The growth-regulating activity of N-(1-naphthyl)phthalamic acid was found to be greater than that of the 3-nitro-1-naphthylphthalamic acid. Effects of both salts were pronounced when the plants were treated as they emerged from the soil **but** the magnitude of the responses decreased when plants at **later stages** of development were treated. Growth responses in the primary leaves were persistent in plants treated at the earliest stage of development.

N-(5-acenaphthyl)phthalamic acid induced growth responses within 24 hours after application of the compound to the stems of bean plants. This response disappeared, however, within 3 days. A second application of the chemical to the stems 4 days after the first treatment caused a slight suppression of terminal growth that resulted in accelerated axillary bud development. Within the next 3 weeks the plants that received 2 applications of the chemical developed almost twice as many pods as did others that received no treatment.

6. Growth-Regulating Properties of the Oxypropanol Alcohol Derivatives of Some Trichloro-Substituted Benzoic Acids.

2,3,6-Trichlorobenzoic acid is a very active growth-regulating chemical when applied either to the stems or leaves of bean plants. In contrast, 2,3,6-trichlorobenzoyloxypropanol induced marked growth responses when applied to the stems of bean plants but proved to be inactive when applied to the leaves. In addition, a mixture of isomers of trichlorobenzoyloxypropanols (2,3,6-; 2,3,4-; and 2,4,5-) also failed to induce growth regulation when applied to the leaves of bean plants. This isomer mixture was, on the other hand, very effective in inducing growth responses when applied to the stems of bean plants. From these results it appears that the oxypropanol form of these trichlorobenzoic acid regulators is either broken down when applied to the leaves of bean into degradation products without growth-regulating activity or the isopropanol form of these compounds is not translocated in effective amounts from leaves to other parts of the plant.

7. New Plant Regulators Tested

The following new growth-regulating compounds were discovered: alpha-azidophenylacetic acid and 3,4-dichloro-alpha-azidophenylacetic acid. These compounds, obtained from Belgium, induced terminal bud inhibition and stem **curvature** when applied to the leaves of bean plants. The chlorinated form was the more active of the two. Neither of these regulators was translocated downward and exuded by the roots of bean plants. Corn plants failed to respond to either compound when these were applied to the leaves. The roots of corn plants failed to exude detectable amounts of **these** regulators when applied to the leaves of the plants. These results indicate that these compounds may be readily degraded into nongrowth-regulating reaction products when applied to corn.

A study of these azido compounds offers a possibility of comparing the effect of an N_3 substitution in the methyl carbon position with similar compounds we have studied earlier involving an alpha-methoxy substitution. N_3 substitution in both the chlorinated and nonchlorinated acids resulted in less active growth regulators than did substitution of a methoxy group on the alpha carbon. Furthermore, the N_3 substitution on the methyl carbon did not result in compounds that exuded from roots following application of the compound to the leaves of plants used in these tests.

8. Absorption, Translocation, and Exudation of 2,3,6-Trichlorophenylacetic Acid

2,3,6-Trichlorophenylacetic acid (Fenac) was absorbed and translocated and induced marked growth responses when applied to one primary leaf or to the first internode of young bean plants. Response was most intense, however, when the chemical was applied to stems rather than to leaves. The compound, or a growth-regulating metabolite of it, was exuded from the roots of bean plants following application to either leaves or stems. When a measured amount of the compound was applied to the stem, a greater amount of the regulator was absorbed, translocated, and exuded from roots than when the same dosage was applied to the leaves. It was then absorbed by roots of a nearby untreated bean plant and moved upward in the stem to partially developed leaves which subsequently became malformed. Leaves of bean plants either degraded the regulator into a less active compound or failed to translocate the applied compound as readily as when it was placed on the stem.

Using corn as a test plant, the stable form of the acid was applied to the leaves. The compound, or a growth-regulating metabolite of it, was absorbed, translocated, and exuded from the roots in sufficient amounts to induce characteristic growth responses when absorbed by roots of nearby bean plants.

9. Growth-Regulation and Root Exudation of 2-Methoxy-3,6-Dichlorobenzoic Acid

2-Methoxy-3,6-dichlorobenzoic acid (Banvel-D) induced terminal bud inhibition and curvature of the stem when applied either to the leaf or stem of young bean plants. The compound was exuded from the roots of the bean plants in sufficient amounts to induce marked growth-regulating responses when absorbed by roots of nearby untreated bean plants.

When the chemical was applied to leaves of corn, no growth inhibition or curvature occurred. However, Banvel-D or a metabolite of it was exuded from the roots of the corn plants and absorbed by roots of nearby untreated bean plants in amounts sufficient to induce terminal bud inhibition.

10. Translocatability of 4-Chloro-2-Methylphenoxyacetic Acid Compared with that of 4-Chloro-2-Methyl Alpha-Propionic Acid

Radioactively-tagged 4-chloro-2-methylphenoxyacetic acid and 4-chloro-2-methyl alpha-propionic acid were obtained to study the translocatability of these compounds. Leaves of bean plants treated with the propionic form translocated 12% of the applied chemical to other parts of the plant. Approximately 14% of the acetic acid form applied to leaves was translocated to other parts of the plant, a difference that is not considered to be significant. No differences were detected in the final pattern of distribution of the two compounds following translocation. These results represent the second case in which a plant regulator with a methyl or methoxy group associated with the alpha carbon in the side chain failed to translocate more readily than did the corresponding unsubstituted acid. It was noted that, in both cases, the side chain was separated from the aromatic portion of the molecule by an oxygen atom.

11. Metabolism of Regulating Chemicals

2,3,6-Trichlorobenzoic acid, a plant growth regulator, exudes from roots of many kinds of plants following application to the leaves. 2,5-Dichlorobenzoic acid has similar growth-regulating properties but this compound, or a growth-regulating metabolite of it, does not exude from the roots in detectable amounts after application to leaves or stems.

Through the use of chromatographic and bioassay techniques, it was learned that 2,3,6-trichlorobenzoic acid was absorbed by leaves of bean plants, then translocated and exuded into aerated water surrounding the roots without chemical change. 2,5-Dichlorobenzoic acid was absorbed and translocated from leaves to the stems where it was detected in an unchanged form. None of this acid, however, was detected in the roots of plants following application of it to the leaves, nor was the acid exuded in detectable amounts from the roots of these plants. These results are evidence that the dichloro acid failed to exude from the roots because it was metabolized into other nongrowth-regulating compounds before it reached that part of the plant.

12. Distribution of Southern Bean Mosaic Virus in Plant Tissue

A method employing frozen-cut, lyophilized, chloroform-fixed sections of infected and noninfected plant tissue in conjunction with a fluorescent antibody stain was used to study the distribution of southern bean mosaic virus in tissue. The presence of a specific fluorescent antibody stain indicated that, after the original infections are established in the epidermal cells of an inoculated primary

leaf, contiguous cells become progressively infected. Staining of the opposite uninoculated primary leaf did not reveal such cell-to-cell progression but, instead, the stain was first observed throughout the mesophyll. Virus progression in the stem above and below the node of the inoculated leaf was from isolated areas of the phloem, to the entire phloem, to the cortex and epidermis, and finally included the xylem parenchyma and pith tissues. In relatively mature roots the virus was present in the phloem, xylem rays, and xylem parenchyma. In very young fruits virus was present in all tissues of the pod but was not observed to be present in the ovule. During a later stage of development the seed coat stained intensely. At this time the cotyledons and developing embryo displayed a slight but specific stain. Preliminary results indicate that some fibers in the xylem and phloem stain specifically, particularly the pericyclic fibers of the phloem.

13. Regulating Compounds that Exude from the Roots of Plants and Their Effect on Nematodes

Some growth-regulating compounds, or their metabolites, exude from roots of plants following application of these compounds to the leaves or stems. 2-Methoxy-3,6-dichlorobenzoic acid, which has a marked growth-regulating effect on bean but only a slight effect on young corn plants, exuded from roots of both kinds of plants in detectable amounts. Cooperative research with Nematology Investigations is under way to determine if the methoxy acid is exuded from roots in amounts sufficient to prevent entry of nematodes into the roots of corn plants following application of the compound to the leaves. Other growth-regulating compounds which are known to exude from roots are also being tested for their possible effect on several species of nematodes.

14. Growth-Retardant Regulators Improve Fruit Production in Holly

Three growth-retardant chemicals, (2-chloroethyl)trimethylammonium chloride (Cycocel), N-dimethylaminomaleamic acid (CO-11), and 2,4-dichlorobenzyltributylphosphonium chloride (phosfon), were applied as soil drenches to young holly plants growing in pots in the greenhouse during July 1961 at about the time fruit buds were forming. The untreated holly plants had not flowered or produced fruit by October 1962. On the other hand, all of the plants treated with phosfon produced flowers and, depending on the individual plant involved, 70 to 100% of these later developed mature berries. Cycocel and CO-11 induced flowering but fewer of the flowers on these plants developed into berries than did those on plants treated with phosfon. Two types of holly were used, Ilex cornuta, Burfordi and a hybrid, I. aquifolium x I. cornuta, var. Nellie R. Stevens. The vegetative growth of Burfordi holly plants was not suppressed by application of the retardant chemicals while the Nellie R. Stevens variety, treated with either phosfon or Cycocel, were about 40% shorter than the controls. CO-11 was not applied to this variety.

15. Further Studies on the Nature and Cause of Internal Sprouting of Potato Tubers

Considerable internal sprouting in experimental lots of potatoes was obtained during 1960-1961 and again during the 1961-1962 storage season. There is no conclusive evidence that internal sprouting is stimulated by the inhibitor 3-chloro-isopropylphenyl carbamate (CIPC). On the other hand, there is definite evidence that less internal sprouting occurred in potatoes treated with increased concentrations of the chemical. The greatest amount of internal sprouting occurred in untreated tubers held at moderately high temperatures of 60 to 65 degrees F., and in tubers that received low dosages of CIPC. There was less internal sprouting both in the treated and the untreated tubers held at 50 to 55 degrees than there was in lots held at 60 to 65 degrees. Storage of the tubers at 70 degrees also reduced the incidence of internal sprouting. These results were obtained with 3 varieties of potatoes - Cobbler, Kennebec, and Katahdin. Cobbler showed earlier sprout growth and a higher percentage of internal sprouts than did the other varieties. This research is in cooperation with Horticultural Crops Branch of AMS.

16. Growth-Retardant Chemicals as an Aid in Breeding of Alfalfa and Carrots

Application of phosfon to soil suppressed vegetative growth of alfalfa and kept lodging to a minimum. The short day-length and dim light conditions of the winter months cause alfalfa plants used in plant breeding research to develop an undesirable amount of vegetative growth and the plants are not able to stand erect. This intertwining of stems makes it difficult to keep the seed heads separated. In preliminary experiments in the greenhouse phosfon suppressed vegetative growth of small alfalfa seedlings most consistently compared with (2-chloroethyl)trimethylammonium chloride (Cycocel), Amo-1618, or N-dimethylaminomaleamic acid (CO-11). The work was extended using varying dosages of phosfon applications to large alfalfa clumps growing in pots in the greenhouse. These larger alfalfa plants were also suppressed in varying degree depending on the dosage level of phosfon applied. At the higher dosage the plants were about 1 foot high when they produced flowers and seed as compared with untreated plants which were 4 to 5 feet high at this time. This research was conducted co-operatively with the Forage and Range Research Branch.

Preliminary research on applications of retardant chemicals to carrots indicates that vegetative growth can be regulated to produce plants with short, sturdy seed stalks to prevent lodging. The most promising results were obtained by soaking the parent carrot roots overnight in aqueous solutions of Cycocel or B-995, a new experimental retardant.

The work is being extended this year to include field-grown carrot seed production. This research is in cooperation with the Vegetables and Ornamentals Research Branch.

17. Evaluation of New Growth-Regulating Compounds

A rapid method of evaluating growth-regulating compounds for tobacco sucker control was developed which made possible the testing of 5,278 compounds in a little over a year. Five-hundred and sixty of the compounds tested retarded bud growth by at least 90% and about 240 inhibited bud growth by 95% without apparent adverse side effects when applied as a 1% mixture in lanolin to the axillary buds. Two-hundred and eleven of the more promising compounds were applied in secondary greenhouse tests as an overall spray. Under these conditions, 42 of these compounds controlled sucker growth to an extent equal to that obtained with maleic hydrazide (MH-30). Of the compounds recently studied, 10 were synthesized in sufficient quantity to conduct large-scale field tests in Puerto Rico. The compounds used in these tests already show some promise. An additional group of 16 promising compounds was supplied for limited field evaluation.

Xanthi-nc, a type of tobacco with small leaves and characterized by rapid elongation of the stem, was used. Growth of the test plants in the greenhouse was accelerated by use of continuous light, intermittent mist, and relatively high temperature during the early stages of development. This work is in cooperation with the Tobacco and Sugar Crops Research Branch.

18. Public Law 480 Research

P.L. 480 project A7-CR-5, FG-In-117 with Calcutta University, India, provides for the study of the presence of growth regulators in plants and plant parts with special reference to the roots of water hyacinth and seeds of tropical plants. Roots of water hyacinth were found to contain a bound auxin and the compound, after hydrolysis, was identified as indoleacetic acid by means of chemical tests and bioassay. Neutral auxins, acid auxins, and gibberellins were not found in extracts of these roots. In preliminary studies auxin activity was observed in an ether extract obtained from immature seeds of Diospyros kaki.

P.L. 480 project A10-CR-29, FG-Is-128 with Hebrew University of Jerusalem provides for physiological studies on plants and plant tissues under drought conditions. The investigator has determined that a hormone capable of inducing plant cell elongation was translocated through tissue segments made up of cells that were maintained in a state of zero turgor. Indoleacetic acid had no effect on the osmotic passage of water through cell membranes in the hypocotyls of sunflower.

P.L. 480 project A10-CR-22, FG-Is-136 with Hebrew University of Jerusalem provides for a study of the occurrence of natural growth promoters and inhibitors in citrus tree organs as influenced by season, age, nutritional disorders, and other factors. Research under this project establishes the fact that indoleacetic acid oxidase was found for the first time to be active in citrus. Location of this enzyme has been established.

B. Plant Virology

1. Purification Techniques

The work on agar gel columns has continued with the development of a calibration technique and the calibration of columns of 1, 2, 3, 4, 5, 6, 7, and 8% granulated agar gels. With this series of calibrated curves, one can determine which gel concentration one should use for the separation of any given sized particulate from contaminating components. One can also use calibrated columns for the determination of particle size on unknowns, and it now appears possible to use them for the determination of diffusion coefficients. The usefulness of agar gel columns has recently been extended for the sorting of rod-shaped particles according to length. In this work standard suspension of tobacco mosaic virus containing the 300 mμ rods, plus various length rods down to unidimensional fragments, were sorted to obtain preparations with better than 95% uniformity of the standard 300 mμ rods, a 2/3 length, 1/6 length, and spheres. Infectivity tests proved that only the standard rods (300 mμ length) were capable of causing local lesions.

A new procedure was developed for the purification of tobacco mosaic virus. This involved the use of EDTA (ethylenediamine tetraacetate) buffers to prevent the pH from dropping below 7.0 during the grinding of infected tissue, the use of charcoal and celite to remove pigmented components, and the use of 8% agar gel column to desalt the virus suspension and get it into 0.001M EDTA of pH 7.5, in which condition aggregation is minimized.

A series of viruses, including tobacco ringspot, southern bean mosaic virus, bushy-stunt virus, tipula iridescent virus, turnip yellow mosaic virus, and sorted standard-length tobacco mosaic virus particles, have been purified following standard methods and refinements in order to obtain suspensions of the highest purity possible. These were furnished to investigators at the University of Virginia for collaborative work involving use of a magnetically suspended centrifuge. A scientist of the University of Virginia has developed a new magnetically suspended centrifuge which makes it possible to centrifuge large molecules or virus particles at very low speeds with a maximum

of rotor stability. This permits the operator to obtain very accurate sedimentation equilibrium conditions with which accurate particle weight or molecular weight determination can be made. A cooperative working arrangement between the Crops Research Division and the University of Virginia has been established to obtain the particle weights of plant viruses with this highly specialized equipment.

2. Isolation of Virus Components.

In collaboration with George Washington University, the structure and bonding within virus particles is being investigated.

In alkaline degradation studies of turnip yellow mosaic virus (TYMV), leading to the formation of empty protein coats, it has been found that the dissociation of protein and RNA is primarily a phenomenon brought about by interference with electrostatic bonding. There is a rapid uptake of OH^- during the alkaline treatment. It can be shown that after this uptake dissociation has taken place. This dissociation reaction can be directed at will to either the formation of RNA + protein shells or RNA + protein dissociation-products (subunits) by working in the presence or absence of certain salts. This phenomenon also points to the fact that the RNA-protein interactions and also the interactions between the protein subunits are primarily of electrostatic nature. It is hoped that studies of this kind (i.e., the nature of the bonding between virus subunits) will eventually lead to a better understanding of the processes of virus dissociation and reassembly in the plant cell.

Previous studies have shown a vigorous reaction between the -SH groups of TYMV and organic mercurials in general and with PCMB (para-chloro-mercuricbenzoate) in particular. The substitution of -SH groups of this virus leads to a loosening of its structure and eventually to collapse of the particles with consequent liberation of RNA. This reaction of PCMB with the protein -SH groups may have potential usefulness in a number of ways.

The mere fact that -SH substitution has consequences for the stability of the particle means that either -SH groups are involved in inter-subunit bonding or that the critical bonding sites are spatially in the proximity of the -SH groups. There have been reports in the literature that PCMB inhibits the uncoating ability and consequently the infectivity of certain animal viruses. These findings are of interest since they point to a possible function of the protein coat in general and of the -SH groups in particular in the uncoating process. With TYMV in particular, the PCMB -SH reaction, leading to disintegration of the virus, may provide new and interesting approaches to the preparation of free infectious RNA.

A preparation of a PCMB-substituted TYMV which, under certain conditions, can be isolated structurally intact but which is highly labile with respect to neutral pH and certain conditions of ionic strength has been developed. The stability of PCMB-substituted virus can, therefore, be directly compared with untreated TYMV, taking titratability in the pH 4.6 - pH 7.0 range as an experimental variable. It is then found that the PCMB treatment has exposed certain titratable groups which in the untreated virus were apparently covered up in the quaternary structure. It is hoped that these titration studies will yield information about inter-subunit bonding. In addition to this, serious attempts can now be made to reconstitute the dissociation products of reactions of TYMV and PCMB.

At this moment the depression of the Optical Density Ratio, 260/240, is being used as a qualitative indication for the extent of PCMB substitution in the virus. However, more quantitative methods are presently worked out in conjunction with a local lesion assay for the virus itself. In this way we hope to carry out studies concerning the effect of PCMB substitution on virus infectivity.

Extracts made from cucumber mosaic virus (CMB)-infected tobacco leaves with pyrophosphate-phenol are more infectious than extracts from similar tissue made with phosphate. Work performed in an effort to find the reasons for this phenomenon showed that the high infectivity of phenol-treated tissue extracts did not originate from free RNA, partially degraded or incomplete virus, or from unstable variants similar to those known to occur in tissues infected with tobacco necrosis virus. Highly infectious CMV-NA preparations could be produced from purified CMV if the virus was suspended in a medium of high ionic strength during phenol treatment. Phenol treatment of virus suspended in media of low ionic strength yielded NA preparations whose infectivity was only a small fraction of that of the virus suspension. The high ratio of NA to virus infectivity appears to be due to an unusual property of the NA, not to low specific infectivity of the virus. Analytical ultracentrifugation of NA preparations showed that they contained two major and two minor components. Density gradient centrifugation of NA preparations disclosed at least three components. Preliminary results show that one of these components is infectious, one is not infectious, and one is probably not infectious.

Work with TYMV is hampered by the unavailability of a reliable local lesion assay procedure. It has been known for a long time that under certain environmental conditions TYMV induces purple lesions in Chinese cabbage. Work is in progress to define these conditions. It was found that, if plants were grown under conditions of low light intensity and if they were subjected to carefully controlled nitrogen stress, they regularly produced purple lesions following inoculation

with TYMV. These conditions are conveniently produced by growing plants in vermiculite by supplying them with nitrogen-deficient nutrient solutions and by performing the bioassays in a growing chamber with artificial illumination.

3. Physiology of Virus Diseased Plants

Infection with potato virus Y and with potato virus X plus potato virus Y causes increases in the concentrations of glutamic acid, glutamine, serine, asparagine, α -aminobutyric acid, and proline as compared with healthy controls. Pipecolic acid was sometimes found in leaves from doubly infected plants but not in leaves from healthy or singly infected plants. Derangements in the concentration of free amino acids and amides were not correlated with symptom production.

4. Serology

Serological studies with purified CMV are in progress. High-titred sera have been obtained. Ouchterlony gel diffusion tests utilizing purified virus and antisera from rabbits subjected to different immunization procedures show that the antibody types which appear are dictated by the route of immunization. The antigen-antibody systems vary from simple to either complex or multiple.

In cooperation with the Vegetables and Ornamentals Research Branch, comparative precipitin tests have been made among antisera for bean virus I and bean virus II developed here and those developed by workers in Germany. The German BV-I and BV-II isolates are very closely related to the local BV-I isolate; however, the BV-II isolates are very distantly related to the German isolate.

In cooperation with Microbiological Associates, Bethesda, Maryland, work has been initiated in an attempt to apply the bentonite flocculation test to the serological detection of plant viruses. The most sensitive serological tests (complement fixation, precipitin ring test) require either highly purified virus or at least clarified plant sap with the danger of nonspecific reaction. Chloroplast agglutination utilizes crude sap but is of very low sensitivity. Using bentonite coated with antibody, we have found that specific flocculation occurs in tests with crude sap diluted as little as 1:10, and in the presence of extremely small amounts of virus. At present it appears that the bentonite flocculation test is more sensitive than any other serological test reported in the past.

5. Movement of Viruses in Plants

It was reported previously that a strain of tobacco ringspot virus (TRSV) was not detectable in the primary leaf opposite to the inoculated one in a systemic host such as Black Valentine bean. Studies with southern beanmosaic virus (SBMV) in the same host revealed that this virus, in contrast with TRSV, was readily detected in the same uninoculated primary leaf.

Various experiments were carried out to gain an understanding of why there is a difference in the translocation of the two viruses between the two leaves. Evidence has been obtained that the two viruses leave the inoculated leaf about the same time and via the same pathway (i.e., phloem), but they do not travel by the same pathway once they reach the petiole on the opposite side of the plant. Most particles of SBMV continue to move via the phloem and thus quickly reach the uninoculated primary leaf. TRSV, in contrast, no longer moves through phloem but moves from cell to contiguous cell, multiplying in each cell, and thus requires weeks to reach the uninoculated primary leaf. The primary leaf, by this time, is highly resistant to infection and multiplication by TRSV.

Previously it had been found that after transport of southern bean mosaic virus in water-conducting vessels, the virus could enter living cells, infect and multiply. An earlier explanation by other researchers of why tobacco mosaic virus could not do this was that it was too large to pass through a living membrane. Data obtained with SBMV indicate that after water transport infection occurs only in young leaves and not in older leaves even though both aged leaves are equally susceptible to mechanical inoculation. Our data agree with a hypothesis suggested by Dr. Flora Scott; i.e., in young leaves, transitional stages between living and dead cells of the xylem occur frequently. Here virus particles would not have to pass through membranes to reach living cells. In older leaves, such transitional stages occur less frequently or not at all. SBMV and other viruses, when restricted to water transport, cannot pass through living membranes, and therefore do not cause infection in the older leaves.

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EFFECTS OF AIR POLLUTANTS ON PLANT GROWTH
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Problem. Damage to plants from air pollutants has been recognized for many years but has increased markedly in recent years because of industrialization and urban development. Early interest in air pollution was largely in the smoke and fumes from industrial wastes and concerned pollutants such as sulfur dioxide and fluorine. Recently a new group of airborne phytotoxicants described as oxidants has been identified that is believed to be primarily derived from reaction products of auto exhaust. This type of air pollution has been recognized for several years as a problem of importance to agriculture in southern California, but only since 1958 has evidence accumulated indicating that it is also a problem in much of the remainder of the United States, affecting the production of tobacco and certain vegetable crops. Weather fleck damage to tobacco, estimated in millions of dollars in some years, has been observed on cigar-wrapper tobacco in Connecticut and Massachusetts. The evidence obtained in 1958 and succeeding years indicates that abnormal concentrations of ozone in the atmosphere are the primary cause of the fleck in tobacco. The economic importance of the weather fleck or "ozone fleck" problem is illustrated by the one-quarter loss estimated by certain Connecticut tobacco growers in their 25 million dollar crop because of a single weekend outbreak of fleck in late July 1959. Losses have been reduced by development and use of resistant varieties, but more basic research is needed to understand the problem so that more effective control methods can be found. Ozone is a very reactive but normal component of the atmosphere in very small amounts. However, certain products of the combustion of fuels in the presence of sunlight are known to increase the production of ozone in the atmosphere and special weather conditions are able to concentrate this and other air pollutants close to the ground. Concentrations appear to be highest near urban centers. Ozone is only one of several possible phytotoxicants that should be investigated to determine the nature of their damage to agricultural crops, to determine tolerance of various species and varieties of plants to pollutants, and to develop suitable control measures.

PROGRAM

The Department's research program to determine the effect of air pollutants on crop plants is very limited. At Cincinnati, Ohio, research is in progress on the morphological, physiological and biochemical changes occurring in plants exposed to toxic atmospheres; the interaction of these changes with other vectors of environment; and development of techniques for using specific pathological symptoms in vegetation and plant organisms for identification of air pollution and estimation of its degree and extent. This research

is conducted by staff members of the Soil and Water Conservation Research Division with funds from the Department of Health, Education, and Welfare.

Research in certain phases of air pollution involving weather fleck of tobacco has been conducted at Beltsville, Maryland, since 1958 when for the first time excellent correlation was found between the appearance of weather fleck on tobacco and high values of ozone. Investigations include the measurement of ozone in the tobacco field and in plant growth chambers; recording the appearance of new fleck and the extent of it; determination of the nature and extent of leaf spotting on selected tobacco varieties in the field following natural fleck development and in greenhouses after exposure to regulated concentrations of ozone in chambers; a study of environmental factors that affect plant growth and susceptibility to ozone injury; and the development of control measures by application of chemicals such as antioxidants. In cooperation with the Public Health Service, readings on oxidant concentration are made and reported as a part of the Washington-Metropolitan Oxidant Network.

The influence of atmospheric fluorides, ozone, and peroxyacetylene nitrate on the carbohydrate metabolism of citrus is being investigated by the University of California at Riverside under research contract.

The Federal scientific effort assigned to research on air pollution totals 4.6 professional man-years.

PROGRESS

A. Physiological Response of Plants

1. Morphological changes. The importance of pre- and post-exposure cultural conditions in causing marked effects on plant sensitivity to irradiated auto exhaust was fully recognized from studies made in the past year. Pinto bean and tobacco were used to indicate peroxyacetylene nitrate (PAN) and ozone injury in the auto-exhaust complex. PAN causes undersurface glazing of Pinto bean, and ozone causes upper surface flecking of the older tobacco leaves. An upper surface flecking on tissue considerably younger than that normally sensitive to ozone has not definitely been determined as caused by either PAN or ozone. A new exposure chamber is planned for ozone fumigations and for other pure components. (Soil and Water Conservation Research Division.)

2. Metabolism. Sulfur metabolism in Chorella vulgaris was investigated for the purpose of understanding the pathway of sulfate reduction as it relates to the problem of sulfur dioxide fixation in plants. Unfortunately, little of value as yet has resulted from these efforts. In the studies of irradiated auto exhaust on nitrogen

metabolism, further progress was made in the development of paper chromatographic standards of non-protein amino acids in tobacco and Pinto bean. (Soil and Water Conservation Research Division.)

3. Growth regulators. Results of the present year showed that sensitivity of some plants to irradiated auto exhaust could be increased or decreased by some plant growth regulators. Phosphon, tributyl-2,4-dichlorobenzylphosphonium chloride, was applied as a soil drench to Pinto bean plants and petunias four days before exposure to irradiated auto exhaust. Two concentrations were used to show partial to complete protection. Petunia and susceptible trifoliolate leaves of Pinto bean were protected by the lower concentration. The effects of phosphon on plants at levels needed to inhibit injury will prevent its use as a good control. Pinto beans sprayed with indoleacetic acid before exposure to irradiated auto exhaust became more resistant than controls. However, sprays of giberellic acid increased plant susceptibility to irradiated auto exhaust. The hypothesis was proposed that indoleacetic acid causes protection by enhancing anti-oxidant properties within the plants. Sulfhydryl groups were implicated in this mechanism. (Soil and Water Conservation Research Division.)

B. Effects of air pollutants on tobacco plants

1. Ozone sampling technology. At Beltsville, continued progress was made in sampling ozone concentrations of the atmosphere. Ozone samples were secured simultaneously by a commercially available ozone meter and by chemical analysis using neutral-buffered potassium iodide solution as the collecting medium in blackened test tube impingers fitted with a 1 mm capillary bore intake. The ozone meter readings invariably were lower and averaged 73 per cent of values determined by chemical analysis.

2. Results of field experiments. The incidence and severity of weather fleck and premature senescence and leaf loss of the Maryland tobacco variety Catterton were shown to be correlated positively with plant population and supplemental irrigation, and negatively with nitrogen rate. These results were obtained in an irrigation, plant spacing, and nitrogen level experiment at the University of Maryland Tobacco Experimental Farm. Fleck and leaf loss were greatest at the 60-pound nitrogen level at spacings of 7260 and 9680 plants per acre and where an additional 2.75 inches of irrigation was supplied. Tobacco yields and estimated dollar value were significantly less in the intensely flecked, high leaf loss treatments.

Bio-assay field experiments showed weather fleck damage to four test varieties occurred in the same differential relation as damage caused by ozone. Nearly equal amounts of fleck were found in tobacco plantings near Beltsville and Upper Marlboro, Maryland,

on three observation dates. No leaf damage appeared on tobacco plants in a field at Beltsville when grown in a plastic chamber supplied with activated-carbon filtered air. In contrast, fleck appeared on plants grown in a similar chamber supplied with unfiltered air.

C. Effects of air pollutants on carbohydrate metabolism in citrus

A large part of the first year's work was devoted to leaf sampling techniques and evaluating methods for carbohydrate analysis. Sugar analysis of lemon leaves from plants receiving variable air treatments (that is, various combinations of the pollutants ozone, fluoride, and peroxyacetyl nitrate) indicated no consistent significant differences in the individual sugar levels between clean air treatment and other treatments. Where activated carbon was used to filter the incoming air and remove oxidant-type pollutants, the average leaf drop per tree was noticeably reduced. Removal of the oxidant definitely increases the average yield per tree. Preliminary attempts to isolate enzymes of the carbohydrate metabolic pathway from citrus leaves were unproductive.

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WEED CONTROL
Crops Research Division, ARS

Problem. The control of weeds is the most critical economic problem in many phases of agriculture. Weeds cause losses in crops, orchards, grazing lands, forests, water supplies, and irrigation and drainage systems. The national annual loss in agricultural production due to weeds has been estimated to be about 3.8 billion dollars. The losses caused by weeds must be reduced by expanding research to find more effective methods of weed control. Improved weed control methods will facilitate farm mechanization, greatly increase production efficiency, and improve the efficiency of the use of human and land resources in agriculture. Research on the life cycles of weeds is critically needed so that any weaknesses in the reproduction, spread, and survival characteristics of weed species can be exploited in developing methods for their control. Therefore, detailed information must be obtained on the nature, growth, and development of weeds. More effective and selective herbicides are needed. Expansion of fundamental studies on the physiological and biochemical responses of weeds and crops to herbicides can provide information on the relation between molecular structure of herbicides and their modes of entrance, movement, behavior, metabolism, persistence, and fate in plants and soils. This information is essential to the development of new herbicides and the efficient safe use of herbicides currently available. Information is critically needed to develop safe and effective methods of using new herbicides that avoid or minimize the accumulation of chemical residues. Information is required on the effect of environment, soil, plant structure, and method and time of herbicide application, and on the effectiveness of herbicides and their persistence in and on crops and soils. Research is critically needed on the integration of chemical and chemical-cultural methods of weed control into mechanized crop production practices, including the development of herbicide-crop rotations that prevent the accumulation of herbicide residues in soils and reduce the risk of injury to subsequent crops in the rotation.

PROGRAM

The U. S. Department of Agriculture has a continuing long-term program in both basic studies and the application of known principles to the solution of weed problems on farms. Research on reducing damage to crops by weeds includes studies of the life histories and growth patterns of individual weeds and the use of cultural methods, biological agents, and herbicides for their control. Weed research includes physiological and biochemical studies to determine the mechanisms involved in absorption and

translocation, the mode and site of action, the effect of environment on plant responses to herbicides and the metabolism and fate of herbicides in plants and soils. Comprehensive studies are made to develop principles, practices, and methods of using herbicides and other weed control techniques in solving regional agricultural weed and brush problems in agronomic crops, horticultural crops, grazing lands, and aquatic and noncropland sites.

All the research on the control of weeds is conducted cooperatively with State Agricultural Experiment Stations with the exception of the research at Beltsville, Maryland, Denver, Colorado, and Mayaguez, Puerto Rico. The work at Denver is cooperative and jointly supported by the Bureau of Reclamation. State Agricultural Experiment Stations usually furnish office, laboratory, and field facilities, as well as funds and other assistance in support of the cooperative research. There is cooperation also with other Federal agencies, including the Bureau of Reclamation and Bureau of Land Management, Department of the Interior, Forest Service of the Department, United States Army Corps of Engineers, and the Advanced Research Projects Agency, Department of Defense, and Plant Pest Control Division of the Department, which ranges from informal contributions to providing funds and personnel in support of weed control research. Industrial companies cooperate in furnishing experimental chemicals, equipment, and funds essential to rapid progress in weed control investigations. Certain private groups, including the National Cotton Council of America and the Central and Southern Florida Flood Control District, furnish financial support of cooperative research on weed problems.

In addition there are two P. L. 480 projects as follows: S9-CR-1 at Montevideo, Uruguay, to find natural enemies of waterhyacinth, alligatorweed and submersed aquatic weeds, and E21-CR-12 at Poznan, Poland, to study the effects of chemical and mechanical control methods on weeds, corn and associated crops.

The total Federal scientific effort devoted to weed control research is 66.5 professional man-years. Of this total, 5.7 is devoted to physiological and ecological basic research at Beltsville, Maryland which is broadly applicable to all crop commodities, and similar studies are conducted at virtually all field locations; 13.7 to aquatic weeds, phreatophytes and noncropland weeds; and 5.0 to research on the behavior and metabolism of pesticides in soils and in plants. In addition, 1.0 man-year in Rabat, Morocco is devoted to a search for insects that may provide biological control of halogeton.

PROGRESS

A. Research Applicable to All Commodities

1. Herbicide Evaluation Studies. The herbicide evaluation program at Beltsville, Maryland, is designed to obtain the herbicidal characteristics of new chemical compounds on a large spectrum of weed and crop test species. The results of this program enable field research personnel to select judiciously new herbicides for inclusion in secondary studies.

More than 50 new herbicides were evaluated in the field on 25 or more test species and more than 100 herbicides were evaluated on 8 test species in the greenhouse during the 1962 fiscal year at Beltsville, Maryland. Each herbicide was applied at two or more application levels as pre- and postemergence treatments.

A preemergence treatment with 4 lb/A of 1-phenyl-4-amino-5-chlor-pyridazone-6 appeared promising for control of broadleaved and grassy weeds in corn, flax, oats, peanuts, peas, safflower, sorghum, Sudan grass, and sugar beets. At higher application rates this compound will function as a soil sterilant.

The N-(p-chlorophenyl)-N'-methyl-N'-isobutynylurea controlled broadleaved and grassy weeds in corn, cucumbers, and peas at an application rate of 4 lb/A. Corn was the only crop with acceptable tolerance to higher rates. As a postemergence treatment this chemical controlled broadleaved weeds but not grasses in cotton, flax, sorghum, and Sudan grass which had acceptable tolerance to the 4 lb/A application rate.

Four uracils were evaluated for their pre- and postemergence herbicide properties. The 5-bromo-6-methyl-3-phenyluracil and 5-bromo-3-isopropyl-6-methyluracil appear particularly promising for control of broadleaved and grassy weeds in flax. The above mentioned uracils and the 5-bromo-3-sec-butyl-6-methyluracil appear excellent for soil sterilization as a pre- or postemergence treatment. The 3-cyclohexyl-5,6-trimethyleneuracil is more active as a preemergence than as a postemergence treatment. As a pre-emergence treatment this uracil controlled broadleaved and grassy weeds in corn and Sudan grass at 4 lb/A.

An analog of ethyl N,N-di-n-propylthiolcarbamate (EPTC), ethyl N,N-diisobutylthiolcarbamate at rates of 4 lb/A showed excellent promise for the preemergence control of broadleaved and grassy weeds in a wide range of crops including alfalfa, buckwheat, field and sweet corn, lima beans, peanuts, peas, snapbeans, squash, and turnips. This compound appears to efficiently control barnyardgrass

and giant foxtail when applied as a preemergence treatment and is well adapted for use at layby after clean cultivation on a large number of crops.

The ethyl-1-hexamethyleneiminecarbothiolate (R-4572) had a wide range of selectivity and controlled both broadleaved and grassy weeds in alfalfa, field and sweet corn, peanuts, and peas. These crops had an acceptable tolerance at the 4 lb/A treatment level. This chemical was observed to control barnyardgrass and giant foxtail. Postemergence application of this compound had no appreciable effect on grassy weeds but control of broadleaved weeds was acceptable in the tolerant crops, oats, peanuts, and turnips.

The omega-(N,N-diethylaminoethyl)chlorophenyl sulfide hydrochloride was relatively inactive as a preemergence treatment but controlled broadleaved weeds in a number of crops as a postemergence treatment. Crops that tolerated this compound were buckwheat, corn, cotton, oats, peanuts, and sorghum.

Four s-triazines appeared promising for the postemergence control of broadleaved and grassy weeds in corn at rates of 2 lb/A. These compounds were: (1) 2,4-bis(ethylamino)-6-ethylmercapto-s-triazine, (2) 2-ethylamino-4-methoxypropylamino-6-methylmercapto-s-triazine, (3) 2-ethylamino-4-methylmercapto-6-n-propylamino-s-triazine, and (4) 2-isopropylamino-4-methoxy-6-methoxyethylamino-s-triazine.

2. Biochemical, Physiological, and Ecological Studies. Scientists at Beltsville, Maryland, have discovered that 2-chloro-N,N-diallyl-acetamide (CDAA) and chloro-substituted benzoic or aliphatic acids interfere with the metabolism of the essential B-vitamin, pantothenic acid. The effect is most readily observed in mutant organisms with limited capacity for production of the vitamin and is thought to be of physiological significance to herbicidal action only for 2,2-dichloropropionic acid (dalapon). Chloro-substituted aliphatic acids inhibit the enzyme which synthesizes pantothenic acid from pantoic acid and beta-alanine. In structure versus activity studies, compounds possessing chlorine substitutions on the carbon adjacent to the carboxyl group inhibited the enzyme by competition with pantoic acid for a site on the enzyme; those compounds lacking this substitution inhibited the enzyme by competition with beta-alanine. In further studies with growing organisms possessing genetic weaknesses in pantoic acid or beta-alanine production, growth was protected against the inhibitors by the growth-limiting substrate rather than by the substrate active at the enzyme level. Thus, metabolic deficiencies may mask the true antimetabolite relation of these inhibitors of pantothenate formation.

At Beltsville, Maryland, the new substituted-uracil herbicides were found to act as inhibitors of photosynthesis. Application of carbohydrates, the products of photosynthesis, partially protected growth of barley seedlings against inhibitions by 5-bromo-3-isopropyl-6-methyluracil (isocil). Unsubstituted uracil is a natural metabolite incorporated into nucleic acid structures; studies with a uracil-requiring mutant organism indicated that isocil does not interfere with uracil metabolism.

Continuation of surfactant evaluations at Beltsville, Maryland, demonstrated that oil-soluble surfactants in oil carriers influenced the activity of herbicides in the same ways that water-soluble surfactants affected activity in aqueous systems. Surfactant enhancement, suppression, or ineffective action was obtained as a function of surfactant concentration. It was found that a single surfactant could exhibit all three types of effects in spray systems in which relative hydrophilic-lipophilic character was varied by changing the carrier or the chemical form of either 2,4-dichlorophenoxyacetic acid (2,4-D) or 4,6-dinitro-o-sec-butylphenol (DNBP). Effects also varied in different test species.

Herbicidal activity of surfactant-amine salts of 2,4-D was evaluated in relation to chemical structure on the amine moiety. Varying the volume rate of spray application demonstrated that a portion of the effect could be attributed to surfactant concentration. Chemical configurations which promoted water solubility were associated with increased herbicidal effectiveness of 2,4-D in aqueous sprays.

Major effort during the past year at Fargo, North Dakota, has been devoted to liaison work between ARS, the architect, the contractor, and GSA during the construction of the Metabolism and Radiation Research Laboratory and in developing specifications for a complex of eight plant growth chambers for investigating the effect of environment upon the metabolism of pesticides in crop plants. Construction of the laboratory was initiated in September 1962, and 35 percent complete. It is expected that the entire Laboratory will be completed by the end of 1963.

3. Behavior of Herbicides in Soil. At Beltsville, Maryland, a soil-solution method and a soil-perfusion method have been most useful for isolation of organisms which effectively metabolize pesticides. Several species of Pseudomonas and Arthrobacter which are effective in the detoxication of dalapon have been isolated and identified. Detoxication of dalapon by these organisms occurs more rapidly in the presence of trace amounts of an additional carbon source. Soil perfusion investigations have revealed that detoxication of dalapon occurs more rapidly in soils having a high organic matter content. In pure culture detoxication occurs

more rapidly when nitrogen is supplied in the nitrate form rather than the ammonium form. Although some organisms appear to be specific in their utilization of dalapon, others are able to utilize other chlorinated aliphatic acids.

Studies on the decomposition of dalapon in soils showed that 7 to 8 days were required for development of a population of soil microorganisms which rapidly decomposed dalapon. Once the population was adapted to rapid metabolism, additions of more dalapon were metabolized within 24 hours. Recent studies suggest that the major pathway of dalapon metabolism is through pyruvate to the Krebs cycle with elimination of carbon-1 as carbon dioxide. A model system is being proposed for the conversion of dalapon to pyruvate in which alpha-chloroacrylate is thought to be the first product of metabolism.

Aspergillus fumigatus, isolated from soil-solution cultures, was effective for metabolism of 2-chloro-4,6-bis(ethylamino)-s-triazine (simazine). Several other soil fungi (three additional genera) thought to metabolize simazine were isolated and identified.

Investigations of the effects of several pesticides on microbial populations in the soil have revealed that isocil caused a decrease in numbers of certain soil-borne plant pathogenic fungi. Preliminary work with several insecticides has indicated that significant quantitative and qualitative differences in soil fungus populations may exist several years following soil treatment with these compounds. Quantitative differences in numbers of actinomycetes or bacteria were not apparent, however. At ordinary application rates dalapon had no significant quantitative effect on soil microbial populations although application of dalapon at higher rates resulted in a significant decrease in bacteria and actinomycetes, whereas the number of fungi increased significantly.

Detoxication of ferric dimethyldithiocarbamate (ferbam) was observed in soil-solution cultures, and an unidentified soil bacterium which can grow on a medium containing this fungicide was isolated.

It was found that several s-triazines volatilized rapidly from soil surfaces under some conditions. Large losses of 2-chloro-4-ethylamino-6-isopropylamino-s-triazine (atrazine) from soil surfaces occurred when soil samples were exposed to temperatures of 95 and 113°F for 5 days. Other s-triazines, particularly 2-chloro-4-diethylamino-6-ethylamino-s-triazine (trietazine) and 2-ethylamino-4-isopropylamino-6-methylmercapto-s-triazine (ametryne) were equally or more volatile from soil than atrazine. Losses of atrazine were greatest from sandy soils and from soils

exposed at 113°F, the highest temperature used. Mixing the herbicides with soil reduce volatility losses.

Losses due to volatility from planchets constitute a serious problem to quantitative studies of C¹⁴-labeled s-triazines. Conversion of atrazine and certain other s-triazines to the corresponding 2-hydroxy forms by hydrolysis in acid solutions prevented loss of radioactivity by volatility.

The effects of several variables on movement of herbicides in soils has been investigated. When water was added in 0.25-inch increments at 30 minute intervals to surface of soil columns after surface application of herbicides, there was more movement of the herbicides than when the same amount of water was added in 1-inch increments. Studies with 2-methoxy-3,6-dichlorobenzoic acid (dicamba) and N,N-dimethyl-2,2-diphenylacetamide (diphenamid) in Hagerstown silt loam revealed that these herbicides readily move upward in sub-irrigated soil columns. Upward movement was directly related to the amount of water that evaporated from the column surfaces. Water applied to the surface of soil columns and then allowed to evaporate from the surface also effected upward movement of dicamba. These studies indicate that evaluations of the relationship between movement of herbicides and rainfall should consider the extent of water evaporation from the soil surface as well as the total amount of rainfall.

Soil adsorption of atrazine was found to be very rapid initially. The rate of removal of the herbicide from aqueous solutions by adsorption to soil suspended in the solution decreased with time but removal from the solution continued up to 16 days. Sterile and nonsterile soils were equally effective. Generally adsorption is a very rapid process, with equilibrium occurring almost instantaneously but these results suggest that adsorption by the soil system is prolonged. This prolonged adsorption is probably due to slow diffusion of herbicide molecules into rather inaccessible sites within the soil particles.

Difficulty has been experienced here and elsewhere in extraction of many herbicides from soils for determination of concentrations and study of herbicide decomposition. Addition of urea or ammonium sulfate to the extraction procedure improved extraction of atrazine from soils with chloroform. Some s-triazines, which were unextractable with chloroform, were recovered (50-70 percent) by use of urea or ammonium sulfate in combination with chloroform. More recently recovery of simazine was improved by continuous methanol extraction, and recoveries of 95 percent were obtained.

B. Weed Investigations - Aquatic and Noncrop Areas

1. Aquatic Weeds

a. Physiological and Ecological Studies. At Prosser, Washington, corn was found more tolerant than soybeans to fenac and dichlobenil in irrigation water. Corn yields were not reduced by postemergence treatments of 10 ppm concentrations of either herbicide in irrigation water. Soybeans were severely injured by postemergence treatments of fenac at 10 and 1 ppm and of dichlobenil at 10 ppm. Injury to soybeans occurred from both herbicides at the 0.1 ppm concentration but yields were not reduced.

Eight of 49 compounds evaluated at Denver, Colorado, showed high activity on submersed weeds in standing water. Four of 5 compounds having high activity in flowing water were materials with surfactant properties. Addition of sulfur to 2,4-D treatments in flowing water increased slightly the activity of 2,4-D. In evaluation of soil-applied herbicides, only one experimental material was very active and showed good residual properties.

The initial flow of water over a section of a canal in Wyoming treated with 20 lb/A of fenac contained a high concentration of the herbicide. The concentration dropped rapidly and after several hours the water was deemed safe for use on crops.

At Denver, Colorado, nodal hypertrophy and vascular disorganization occurred in sago pondweed grown in soil treated with fenac at 5 lb/A. Plants were affected early in growth and translocation of herbicides was severely restricted.

In controlled environment studies at Denver, Colorado, plant injury increased linearly with aromatic solvent concentration, plant injury increased with plant age and was directly correlated with the temperature of water used during herbicide application. Sago pondweed grown under controlled environmental conditions increased linearly in dry weight up to about 20 mg/day at 30 days. Dry weight of tubers decreased from 20 to 8 percent in 15 days. Dry weight of shoots reached a stable value of about 8 percent after 10 to 12 days.

In herbicide evaluation studies at Fort Lauderdale, Florida, dichlobenil and one other experimental material showed promise as possible herbicides for controlling alligatorweed. In carbohydrate reserve and utilization studies, the low point in carbohydrate reserve in untreated alligatorweed occurred in July. Total darkness for 5 weeks reduced the carbohydrate content by 48 percent. Bud development in alligatorweed was found to be light dependent and the fresh weight of this species increased with increases in light intensity from 100 foot-candles to full sunlight. Maximum growth of southern naiad occurred at light intensities of 400 to 700 foot-candles.

Water in some Florida canals contained various concentrations of tannins which influence the light intensities found at various depths. A close correlation between absorption of light and submersed weed growth was apparent.

Ten to 14 days were required for the dissipation of 6,7-dihydro-dipyrido (1,2-a : 2',1'-c) pyrazidiinium salt (diquat) from nonflowing water of lakes treated in Florida and Georgia. Fish food was not affected and plankton recovered within one week of the treatment.

Water analyses of 11 water systems in California indicated that abundant growth of submersed aquatic plants may be strongly correlated to adequate amounts of either calcium or magnesium, or both. The cation exchange of 13 canal-bottom soils was found to increase as both organic matter and colloidal clay components increased, similarly to that in well drained soils.

b. Control Studies. At Bozeman, Montana, fall application of fenac at 20 lb/A to the canal-bottom soil effectively controlled sago pondweed. The granular formulation of fenac was superior to the amide and liquid sodium salt formulations. Control of pondweed with spring applied endothall was equal to fall applied fenac and better than any other herbicide applied in the spring.

Three formulations of fenac at rates up to 20 lb/A were applied to a canal bottom at Prosser, Washington, for control of pondweeds. Control was not obtained with any treatment. The poor results may have been due to frozen soil at the time treatments were made. Aromatic solvent containing 2 percent emulsifier performed better than the same solvent containing 1 percent. Differences in performance were less striking with decreasing weed infestations.

Soil application of fenac at rates up to 20 lb/A in Wyoming canals were not as effective as similar treatments made the preceding year. Sago pondweed infestations showed midseason stand reductions of 39 to 78 percent from treatments with 10 and 20 lb/A of dichlobenil. The poorer results in 1962 may have been due to insufficient precipitation before irrigation water was turned into the canals.

A dense stand of American pondweed in a small lake in Colorado was completely eliminated by treating a portion of the ice over the lake with fenac granules. General distribution of the herbicide throughout the lake appeared likely and the concentration of herbicide in the total volume of water was calculated at 0.9 ppmw.

In Florida, paraquat applied at 3 to 9 lb/A was very effective on floating torpedograss but less effective when this weed was growing on banks. Diquat and paraquat continued to look promising for control of southern naiad; and granular fenac controlled this weed in canals for 9 months. Addition of fenac to 3-amino-1,2,4-triazole--ammonium thiocyanate (amitrole-T) increased the effectiveness of the latter herbicide on waterhyacinth. Results in 1962 with amitrole-T on waterhyacinth and with silvex on alligatorweed were sufficiently promising to justify plans for initiating large-scale field testing by the U. S. Army Corps of Engineers in Florida in the fall of 1962 and spring of 1963.

Good progress was made during 1962 in P. L. 480 Research Project, "Natural enemies of aquatic plants," S9-CR-1, FG-Ur-107, Uruguay. Natural enemies discovered included six insects which were found feeding on alligatorweed (Althernanthera spp.) and 20 insects and two diseases which were observed causing significant injury to waterhyacinth (Eichhornia spp.) and other genera in Pontederiaceae. The natural enemies discovered appeared to be more effective in controlling waterhyacinth and Pontederia in Uruguay than did the insect enemies of alligatorweed.

At Clarkedale, Arkansas, exposure of waterprimrose to water containing an ester of 2,4-D at 2, 10, and 25 ppm after which the treated water was replaced with fresh water resulted in a 100 percent topkill at all concentrations, 10 percent regrowth at 2 ppm, no regrowth but reinfestation from seed in the soil at 10 ppm, and no regrowth or germination of soil borne seed at 25 ppm.

2. Phreatophytes

Of several environmental factors measured at Los Lunas, New Mexico, the concentration of sodium in ground water appeared to be the most closely related to rate of growth of saltcedar. Solar radiation appeared to be correlated with carbohydrate storage in saltcedar roots. The growing season in 1962 was abnormally dry with less than half of the normal precipitation during the first 8 months.

Dicamba was the only granular formulation of herbicide that produced visible injury to saltcedar. Only fenac produced substantial injury to saltcedar from spray applications to the soil surface. Basal sprays of the butoxyethanol ester and oil-soluble amine of silvex were superior to basal sprays with other herbicides. In combination treatments of burning, mowing and spraying with herbicides, silvex applied at 4 lb/A was more effective following burning or mowing than on undisturbed plots.

Broadcast applications of fenuron pellets at 15 lb/A eradicated a mature stand of saltcedar in Wyoming and were superior to similar rates applied in 1-foot-wide bands 5-1/2 or 11 feet apart. Spring applications were more effective than winter ones.

3. Ditchbank and Other Noncropland Weeds

a. Physiological and Ecological Studies. In Wyoming, a vigorous stand of grass seedlings developed from a broadcast seeding of Kentucky bluegrass, redtop, meadow foxtail, and western wheatgrass on canal banks where 70 percent of existing Carex was controlled the previous year by two applications of amitrole at 10 lb/A, dalapon at 20 lb/A, or 2,4-D amine at 80 lb/A.

In studies at Prosser, Washington, at least 5 years of storage in fresh water were required to reduce the viability of Austrian peaweed to zero. Seeds of ladythumb showed 35 percent firm seed and 24 percent germination after 5 years' storage.

b. Control Studies. Dimethylamine salts of 2,3,6-TBA, polychlorobenzoic (PBA) and dicamba acids were most effective in eradicating Russian knapweed at Prosser, Washington. Single high rates were more effective than repeated lower rates.

The most efficient treatment for control of reed canarygrass at Prosser, Washington, was amitrole-T at 4 lb/A. Fall applications of herbicides plus retreatments were more detrimental to canarygrass than initial treatments in spring followed by retreatments. Encroachment of canarygrass in competition with more desirable grasses was observed to continue during 1962.

At Bozeman, Montana, ten repeated cultivations every 20 days eradicated 10 ecotypes of Canada thistle after three cultivations in the second season. Some ecotypes continued to be more resistant than others to repeated applications of amitrole and 2,4-D. Dicamba was superior to other herbicides tested for control of Canada thistle.

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NEMATODE IDENTIFICATION PHYSIOLOGY, AND CONTROL
Crops Research Division, ARS

Problem. Plant-parasitic nematodes occur in all soils used for growing of crops and plants and attack all kinds of plants grown for food, forage, fiber, feed, or ornamental purposes. Damage is partly due to the direct effect of the nematodes feeding on the roots or other parts of the plant, and partly due to invasion of nematode-damaged tissue by bacteria, or fungi. Nematodes have been found to be vectors of several plant viruses. Economic damage to crop plants by nematodes varies greatly from field to field, but data obtained from experimental plots commonly demonstrate crop yield reductions of 25% and reductions in excess of 50% are often found. Nematodes can be controlled by crop rotations, cultural practices, chemicals and biological agents, and damage can be avoided by growing nematode-resistant crop varieties. All of these control methods involve a certain amount of expense and none are as efficient as desired. There is urgent need for fundamental research on the taxonomy, physiology, ecology, and relationships of nematodes to bacteria, fungi and viruses. There is also need for extensive research to develop less expensive, practical, and more efficient methods of controlling nematodes on annual crop plants, and for the development of methods of controlling nematodes on producing orchards, citrus groves and vineyards, and on nursery plants.

PROGRAM

The Department has a continuing long-term program of basic and applied research on nematodes and their relations to crop losses, and on development of practical control methods. Basic research on the taxonomy and physiology of nematodes is conducted at Beltsville, Maryland, and at field stations in various states.

In addition there are three P.L. 480 contracts as follows: Poznan, Poland for basic research on the nature of plant resistance to nematodes; La Molina, Peru for research on the golden nematode of potatoes; and Talawakelle, Ceylon for research on the root-lesion nematode.

The Federal scientific effort devoted to research in this area in FY 1963 totaled 21.5 man-years. Of this total 5.5 is devoted to basic research on nematodes.

PROGRESS

A. Taxonomy. Anomalies observed at Beltsville, Maryland, in the nematode specimens being identified as soybean cyst nematodes (Heterodera glycines) in connection with the quarantine on this species led to an investigation of the situation in the field. This investigation revealed that nematodes from Union County, North Carolina were a new species of Heterodera which attacks lespedeza heavily and does not attack soybeans. "Off-type" specimens from Virginia and other parts of North Carolina are still under investigation to determine if they are another new species or variations of the soybean cyst nematode.

Work has continued on the United States Department of Agriculture Nematode Collection which now contains a total of 531 cataloged entries involving 581 permanent slides, 373 vials and 50 museum jars, plus the magnificent collection assembled by Gerald Thorne, a former employee. In addition, work on experimental taxonomy has been started with an investigation of the stability and adaptability of four Heterodera species, a study of the need for males in reproduction of Heterodera cyperi, and host tests of the clover cyst nematode Heterodera trifolii.

During the year, ten new species of plant-parasitic nematodes were discovered, 5 from the United States and the others from foreign countries. The hop cyst nematode (Heterodera humuli) was identified from the United States for the first time, being found in Pierce County, Washington.

The life history of a sheath nematode, Hemicriciconemoides chitwoodi, was worked out.

B. Physiology. At Beltsville, Maryland, for the first time, the presence of an enzyme capable of degrading some type of cellulose has been demonstrated in plant-parasitic nematodes. This is of scientific interest because it helps explain how nematodes get their food from plants. It is of practical interest because it suggests trials of enzyme-inhibiting chemicals as a means of protecting plants against nematode attack, and because it suggests that the presence of enzyme-inhibitors may be an important difference between nematode-resistant and nematode-susceptible plants.

Investigations of the relationships of root-knot nematodes and the fungus, *Fusarium*, at Auburn, Alabama have shown that the fungus enters cotton roots through decaying tissues previously injured by the nematodes. The *Fusarium* was not found in nematode-infected roots 8 days after planting, but was found in abundance 11 days after planting, growing profusely in decaying cortical cells and in the xylem elements and giant cells, but less abundantly in healthy cortical tissue, and not at all in healthy phloem and cambium. In other words, the invasion of the roots by the fungus was not through wounds made by the entry of the nematodes into the roots, but more closely associated with the formation of giant cells and decay of the roots long after the entry of the nematodes.

C. Control. Studies of a fungus of the genus *Catenaria* at Baton Rouge, Louisiana for biological control of nematodes have progressed to the field experiment stage. This fungus invades nematodes and kills them rapidly, but is of little value if simply added to the soil. For the fungus to be most effective, some material which will stimulate growth is needed in large quantity. Filter press cake, a by-product of cane sugar mills and molasses have been tried. Results were encouraging, and further trials will be conducted.

During the past several years, some Dutch research on control of nematodes by planting marigolds has received considerable publicity and has been reported with considerable exaggeration in popular journals and a recent popular book. Experiments in Talawakelle, Ceylon under P.L. 480 Project A5-CR-1, have shown that the reduction of nematodes in tea plantations by planting marigolds is no more than would be expected from planting of any other cover crop immune to the nematode affecting the tea. Nematodes in the tea roots were not killed by secretions from the marigold roots, as has been claimed.

If nematodes can be prevented from producing progeny, they can be effectively controlled. Laboratory tests of several chemicals known to be capable of causing insect sterility, showed that some of the chemicals might be useful in nematode control. When applied as drenches to soil around tomato plants growing in pots at Orlando, Florida, reduction in root-knot infection of the plants was as high as 99%, with little injury. This is a promising new approach to nematode control, particularly of perennials. However, materials so far tested are far too hazardous to humans to justify their large scale testing.

In the past few years, numerous reports of the value of sugar for control of nematodes has been widely circulated throughout the world. Tests at Salinas, California have shown that sugar is of no value for control of the sugar beet nematode (Heterodera schachtii), since only a small portion of the nematodes in cysts were killed by concentrations which would require application of more than twice as much sugar as can be produced by an acre of sugar beets. Tests at Jackson, Tennessee with the soybean cyst nematode (Heterodera glycines) gave similar results.

In field experiments at Tifton, Georgia some of the new nematocides and combinations of the older ones were compared. A combination of one part technical DBCP (1,2-dibromo-3-chloropropane) with 18 parts of D-D Mixture (dichloropropene-dichloropropane), and a combination of one part DBCP and 4 parts of an 83% EDB (ethylene dibromide) mixture gave satisfactory control of root-knot nematodes, as would be expected, these having been in general use separately. Methyl isothiocyanate with D-D Mixture was also effective. Among the newer materials, Zinophos (a phosphorothioate compound), and tetrachlorothiophene were also effective, though slightly phytotoxic to all crops tested at high rates of application.

The normal action of nitrifying bacteria in the soil is decreased for several weeks after application of several of the standard nematocides. Experiments at Tifton, Georgia have shown that this effect can be minimized by application of 50% of the total nitrogen of the regular fertilizer as nitrate.

Studies on the nature of resistance of plants to nematodes in Poznan, Poland under P.L. 480 Project No. E21-CR-5 have shown that the golden nematode of potatoes (Heterodera rostochiensis) enters resistant potato varieties in smaller numbers than it enters susceptible ones. The nematodes which do enter either fail to develop to the adult stage or become males.

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EPIDEMIOLOGY AND MYCOLOGY OF PLANT DISEASES AND MUSHROOM CULTURE
Crops Research Division, ARS

Problem. Despite considerable progress in controlling many diseases of plants by chemicals and by breeding resistant varieties, diseases still cause annual losses estimated in billions of dollars. Fundamental to all applied aspects of disease prevention and control is the knowledge of the identity of the causal organism, its life cycle, epidemiology, peculiarities, ecology, and relationships to other organisms. Broader taxonomic coverage of the enormous fungus field (100,000 described species) is urgently needed to provide authoritative identifications of plant pathogens and basic research on their characteristics and classification. More study is needed on the factors involved in epidemic outbreaks of plant diseases, and means of appraising the losses in order that maximum use can be made of disease forecast methods and the warning service already developed to facilitate prevention and control programs against plant disease epidemics in the various States. For diseases caused by soilborne pathogens that have been especially difficult or impossible to control by chemicals, breeding, and crop rotation, the discovery of the nature of, and means of maintaining, a favorable microbiological balance in the soil seems to be the only solution and requires ecological studies of the soil organisms and research on the physiology and biochemistry of soil microorganism-plant pathogen relationships.

PROGRAM

The Department research is conducted at five locations. Identifications of plant pathogens, edible and poisonous mushrooms, puffballs, and wood-rots are made at Beltsville, Md., where the fungus herbaria of the Department are maintained. Specimens are constantly being added, loaned, studied and exchanged. Fungus nomenclature is checked for all Division manuscripts. Special taxonomic studies are made on parasitic leaf and stem fungi, wood-rots and certain lower fungi that are of industrial importance with regard to grain storage. Wood-rotting fungi of the Mississippi Delta are being studied in cooperation with the Forest Service.

Research on biological control of soilborne diseases conducted at Beltsville, is intended to point the way to finding biological controls for the otherwise largely uncontrolled diseases that are not economically amenable to chemical control. Studies ultimately will include finding practical soil management, rotational, mulching, and other methods of decreasing populations of pathogenic organisms. Studies on nematode-capturing fungi are included.

A contract with the Department of Agriculture, Annamalai University, Annamalainagar, India, provides for the study of the microbial populations of various soil types, isolation of antibiotics, and the use of the derived antibiotics in disease control. Its duration is 5 years, 1962-1966, and involves expenditures of P. L. 480 funds amounting to \$39,057 in Indian rupees. A similar contract with the Sri Venkateswara University, New Delhi, India, provides for the study of the effects of antibiotic substances and antagonistic soil micro-organisms on important plant pathogenic fungi. The contract is for 5 years, 1963-1967, and involves P. L. 480 funds with a \$9,113 equivalent in Indian rupees.

Department scientific effort devoted to research in this area totals 13.0 professional man-years. Of this total 3.0 is devoted to identification and classification and 3.0 to biological control of soilborne diseases.

PROGRESS

A. Identification and Classification of Fungi

Continued attention was given during the past year to the fungus herbarium acquired from the Missouri Botanical Garden and the final 9746 specimens, representing about one-eighth of the total number received, have now been added to the National Fungus Collections. In addition, another 6896 specimens from various sources were added and bring to 676,828 the number of accessioned specimens in the several herbarium units making up the collections. An especially noteworthy addition during the year was the entire collection of Monochaetia, Pestalotia, and related fungi accumulated and studied by E. F. Guba over a period of almost forty years. Included are the microscopic slides and notes which, together with the specimens, formed the basis of Guba's important recent monograph of these fungi.

The demand for the loan of specimens, exchanges, and the identification of plant disease and other fungi continued at a fairly high level. Almost 2900 specimens were included in 87 loans to specialists of the U. S. and 10 foreign countries. Cooperative work with the Plant Quarantine Division has continued. The mycologist assigned to this work studied 1669 specimens received from plant quarantine inspectors. Miscellaneous identifications of fungi by staff members totaled 272 reported by letter and numerous ones of local origin for both technical workers and the general public. Some 4700 fungus specimens were received from 37 institutions throughout the world on an exchange basis and 4365 duplicate specimens were dispatched in continuation of exchange agreements.

The results of basic research on numerous groups and aspects of fungi have been published, including further studies on the "pecky" cypress fungus and its occurrence in Japan and Formosa; the taxonomic revision of two species of the Myxomycete genus, Arcyria; the discovery of a new species of Elsinoë in Panama; the report of a case of human poisoning from the puffball, Scleroderma cepa; and the production by fermentation techniques of the new chemical, "ramulosin," discovered jointly with members of the Northern Utilization Research and Development Division. Two United States patents have been issued for the new chemical, which was found initially to be produced by a non-sporulating strain of Pestalotia ramulosa. A third patent is pending.

Work on the wood-rotting fungi of Mississippi has continued in cooperation with the Forest Disease Laboratory of the Forest Service and has been directed primarily toward detailed microscopic studies of fungal anatomy and cultural characteristics of the specimens collected in Mississippi. As the result of continued literature review, together with the anatomical studies, all research notes have been organized to conform with the most recent authoritative taxonomic criteria. In recent months a specimen-by-specimen check of the National Fungus Collections was initiated to record pertinent specimens for further study. With one-quarter of the collections currently surveyed, some 800 additional specimens pertinent to work of the project have been uncovered.

Classification studies on the ascomycetous genus Dimeriella have begun with an extensive literature survey. Already the original descriptions and other important taxonomic information have been compiled for most of the fifty published species and more than twenty species of closely related genera. A paper concerning comparative studies of five species of Dimeriella is in press and a systematic study of the pertinent specimens in the National Fungus Collections is underway.

Studies on Cambodian fungi have continued in informal cooperation with a member of the U. S. Agency for International Development. A preliminary check-list of Cambodian plant diseases has been issued and a supplement to it is expected to result from studies still in progress.

The nomenclatorial catalogues have been increased by 2945 entries and records have been made of 137 new genera, including the records being assembled for a proposed revision of the standard work in the Genera of Fungi and for an annotated listing of fungi named by Spegazzini, a South American mycological pioneer. The fungus names used in 128 manuscripts for publication by Department workers were checked for conformity to the International Code of Botanical Nomenclature.

In continuing the cataloguing of foreign and domestic literature on the occurrence and distribution of plant diseases and fungi in general, 21,717 new entries were inserted in the host, fungus, and geographic card catalogues which now contain well over 1,000,000 cards.

B. Biological Control of Soilborne Diseases

1. Relation of DL-homocysteine, DL-methionine, and methyl donors to Aphanomyces root rot control of peas. Previous work on nutrition of Aphanomyces euteiches showed that the form of sulfur (S) profoundly affected growth and sexual reproduction of the fungus. S-containing amino acids, especially methionine, were most favorable for high mycelial yields and production of oospores. Subsequent studies on the effect of various S sources on the development of Aphanomyces root rot of peas grown in nutrient solution and inoculated with the fungus led to the finding that methionine was unable to prevent disease development completely even though it was in no way detrimental to the pathogen. Several compounds lacking S but containing methyl (CH_3) groups, such as norleucine, norvaline, O-methylserine, and beta-methylaspartic acid, were effective in reducing disease severity. As a result of these studies the hypothesis was advanced that the CH_3 group of methionine rather than its S atom was responsible for disease control.

Several CH_3 donors (betaine, choline, dimethyl-betapropiothetin chloride, S-methylmethionine) were tested and found ineffective. Of a number of compounds lacking CH_3 groups only DL-homocysteine, a precursor of methionine, completely controlled the disease. DL-homocysteine itself like methionine was not toxic to the fungus. Both compounds increased zoospore germination and mycelial growth of the pathogen. DL-homocysteine was taken up by pea plants from nutrient solution and converted internally to methionine. The methylation of DL-homocysteine to methionine in pea tissue occurred without an exogenous source of labile CH_3 groups. The fact that DL-homocysteine was converted to methionine endogenously led to the conclusion that it was methionine, rather than homocysteine itself that prevented pathogenesis.

2. Experimental control of Aphanomyces root rot of peas with CH_3 -containing amino acids. Experiments in the greenhouse with unsterilized soil to study the effectiveness of several amino compounds against Aphanomyces root rot of peas were continued. Effective control of the root rot was obtained with 2 to 3 drenches of methionine or norleucins (at 70 ppm each) applied within a few days before or after soil infestation with zoospores. Total concentrations higher than 210 ppm were phytotoxic to peas. Methionine was more phytotoxic than norleucine at equal concentrations. One drench at 30 ppm reduced root

rot, but reduction was less pronounced than at 70 ppm. Root rot was not reduced if more than 3 days elapsed from soil infestation to the first drench application. Methionine and norleucine at 140 ppm as side-dressings, with or without fertilizers, applied 2 to 3 days before or 1 day after soil infestation reduced root rot greatly. The effectiveness of the amino acids was increased by combining them with fertilizers. Applications of methionine or norleucine below the seed or at the same level as the seed at planting time were less effective than drenches and side-dressings. In soil "naturally" infested with the pathogen single drenches or side-dressings with methionine or norleucine at 140 ppm effectively controlled root rot, but methionine was injurious to the plants.

3. Comparative effectiveness of organic amendments, nitrogen and fungicides in reducing activity and survival of *Rhizoctonia solani*. The saprophytic activity of *Rhizoctonia* was reduced by the decomposing action of green and mature organic amendments, especially when the carbon/nitrogen (C/N) balance of the amendments was adjusted with supplemental nitrogen. Permeation of artificially and naturally infested soils with CO₂ air mixtures showed that the pathogenic activity of this fungus was more sensitive to CO₂ than its saprophytic activity, and the latter was more sensitive to CO₂ than the survival ability.

Cellulose powder, oat straw, and soybean hay enriched with NH₄NO₃ to produce C/N ration of 60, 30, and 10 respectively, were just as effective as the fungicide PCNB and more effective than the experimental fungicides H 3944 and CP 30249 in reducing the saprophytic activity of *Rhizoctonia solani* in artificially infested non-sterilized soils 4 weeks after incorporation. One week after incorporation only oat straw and the 3 fungicides at 50 and 100 ppm of the active ingredient reduced *R. solani* activity appreciably. The effectiveness of CP 30249 and PCNB in reducing the saprophytic activity of 5 *R. solani* clones in soil depended on the fungicide and inoculum concentrations and on the different sensitivities of the clones. Oat straw with C/N ratios of 30 and 80 was more effective in reducing the survival of *R. solani* in pre-colonized substrate segments than oat straw with C/N ratio 10 and PCNB at 25 and 50 ppm. High de-colonizing ability of oat straw with low N content was associated with increased numbers of actinomycetes and bacteria in soil. The sensitivity of *R. solani* to amendment decomposition and fungicidal action was greater before the fungus saprophytically colonized the substrate than after it became established within the substrate.

4. Microbial antagonism in bean rhizosphere as affected by oat straw and supplemental nitrogen. Ground mature oat straw (C/N ratio 83) or oat straw enriched with NH_4NO_3 to produce C/N ratios of 30 or 50 incorporated in soil increased numbers of bean rhizosphere microorganisms antagonistic to Fusarium solani f. phaseoli, Rhizoctonia solani, Thielaviopsis basicola, and Verticillium albo-atrum. High N application (200 ppm from NH_4NO_3), either alone or with oat straw (to produce a C/N ratio of 10), substantially decreased numbers of bean rhizosphere antagonists. Both nitrate N and ammonium N suppressed numbers of antagonists, but the suppression, with few exceptions, was more pronounced with the former than with the latter N ion. Suppression by N was greater in very acid soil (pH 4.7) than in acid and alkaline soils. Suppression of rhizosphere antagonists could not be attributed to changes in soil reaction. The majority of antagonists from rhizosphere of beans grown in the very acid soil were true bacteria, whereas the majority of those from beans grown in slightly acid and alkaline soils were Streptomyces spp. The greatest amount of lysis of mycelium of the 4 assay fungi was caused by soil amended with oat straw (with or without N) and the smallest amount by autoclaved soil or by NH_4NO_3 added alone to the soil. Spores of the assay fungi were not lysed under any conditions in these tests.

5. Control of plant pathogens by antibiotics. As part of a contract with The Annamalai University in India, a crude extract isolated from a species of Streptomyces was found to have an inhibitory effect on four species of Helminthosporium. Additional tests were made to find antibiotic substances antagonistic to other plant pathogens, particularly Pseudomonas species.

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REPLACEMENT CROP INTRODUCTION AND EVALUATION
Crops Research Division, ARS

Problem. American Agriculture is characterized by an ability to meet changing demands of our modern society. In crops research, the betterment of agricultural production is brought about by the development of improved varieties with greater resistance to insects, diseases, and climatic hazards, increased adaptation, and higher quality, and by the discovery of new and replacement crops. An important part of this program is the searching for and introduction of plants to provide the basic stocks essential to solving the problems inherent in crop improvement and diversification. There is a need to systematically survey the world's plant resources and develop precise information on species potentials through economic botanical research collated with the needs of industry and agriculture for new raw materials, and to introduce promising new plants through either direct exploration or international plant exchange as required to meet specific crop improvement problems.

Preliminary evaluation of crop breeding stocks and potentially new crops for production in the United States is needed on a strong nationally coordinated basis. The broad range of established crop plants and those in early stages of development require that the many plant introductions involved in crop improvement receive a careful preliminary evaluation to provide crop researchers at Federal and State experiment stations with the most desirable germ plasm. In the development of potentially new crops, research is needed on acclimatization, cultural evaluation, population studies, and finally, advanced agronomic evaluation, correlated with chemical and industrial assessment.

Finally, there is a need to maintain and preserve our diverse wealth of crop germ plasm through the facilities of the National Seed Storage Laboratory and through the inventory and the maintenance of asexually propagated collections either at Federal and Regional Plant Introduction Stations or other similar institutions in the United States.

PROGRAM

The nature of this program is to undertake the introduction, preliminary evaluation, and preservation of all economic plants for the development of a strong yet diversified agricultural program in the United States. Specifically, this program involves the survey of the plants of the world for those species, economic or otherwise, which are essential to solving the current problems of agriculture,

the procurement of the desired germ plasm through exploration and international exchange and quarantine propagations, the evaluation of the introductions either as breeding stocks, as potential new crops, or for conservation and land restoration purposes, the increase and distribution of such materials to Federal, State, and private research agencies, and the preservation of valuable germ plasm either as seed or as vegetative stocks. The research involves agronomists, botanists, horticulturists, plant pathologists, and physiologists who are engaged in both basic and applied studies that will provide plant scientists and others with documented germ plasm basic to agricultural research.

Plant introduction is undertaken in both foreign and domestic fields, either through direct exploration or international exchange, to provide materials for breeding stock evaluation and for new crop development. Taxonomic and economic botanical research on world plant resources, development of national inventories of introduced stocks, coordination of foreign and domestic plant collecting, and botanical assessment of the results of crops/utilization screening programs are conducted at Beltsville. Cooperative arrangements with the National Institutes of Health provide for collection of plants for anti-cancer activity screening, with Longwood Gardens for ornamentals, and the four regional projects, NE-9, NC-7, S-9, and W-6 for domestic explorations for crop breeding stock. Public Law 480 projects in India, Israel (2), Korea, Pakistan, Turkey, Spain, Yugoslavia, Colombia, and Uruguay provide for the collection of plants for utilization and agronomic evaluation for potential new crops. The needs of AID missions abroad for research materials and foreign distribution of germ plasm of cacao, coffee, and rubber are handled at Beltsville by cooperative agreement.

New crop evaluation includes two main areas of research: (1) evaluation of breeding stocks and (2) development of chemurgic crops. Activities in the evaluation of crop breeding stocks are centered at Beltsville with programs at four Federal Plant Introduction Stations and programs under four Federal/State Regional Cooperative Projects. Maintenance of germ plasm is at Fort Collins, Colo., and Federal Introduction Stations.

At the Federal Introduction Stations, Glenn Dale, Md., Savannah, Ga., Miami, Fla., and Chico, Calif., horticultural and specialty crop introductions undergo preliminary evaluation, involving observations for specific characters needed in varietal improvement, development of potential new or improved rootstocks, quarantine propagation and indexing for viruses, and the maintenance of collections of important foreign varieties that are continually requested by breeders for specific research activities. At the Regional Plant Introduction Stations, Geneva, N. Y., Experiment, Ga., Ames, Iowa, and

Pullman, Wash., field crops, vegetables, and other seed-propagated plants are given preliminary evaluation and increase to provide research workers at Federal and State experiment stations with a broad base of outstanding parental stocks. Regional station pathologists screen the introductions for disease tolerance. In some instances, Federal, State, and private breeders cooperate in the early evaluation of crop plant introductions. Material resulting from the Federal and Regional programs that shows breeding potential on the basis of assessment by the four Regional Program Coordinators and the national leaders at Beltsville, Md., is made available to all research workers in the United States. Needs for additional breeding stocks are assessed by the research leaders at Beltsville and these requests become the basis for activities in plant introduction.

The preservation of valuable seed germ plasm, including important plant introductions, selected breeding lines, and new crop varieties, together with research on problems affecting long-term seed viability, is undertaken at the National Seed Storage Laboratory, Fort Collins, Colo. National inventories of asexually-propagated breeding stocks held at Federal and State experiment stations are developed at Beltsville, from data supplied by individual research workers.

Species of plants determined to have important chemical and physical properties as a result of plant introduction research undergo developmental studies at both Federal and State experiment stations. Research is undertaken in several agronomic areas, including climatic and edaphic studies, population evaluations for selection of outstanding parental lines, preliminary cultural and seed increase studies, and the development of agricultural practices including propagation, field establishment, insect and disease assessment, fertilizer requirement, and harvesting methods. Initial phases of this research are conducted at locations providing diversity in environment, such as Federal Stations at Glenn Dale, Md., Savannah, Ga., Miami, Fla., Chico, Calif., Cheyenne, Wyo., and Mayaguez, P. R., and at the Alabama, Louisiana, Nebraska, North Carolina, Montana, Texas, and South Carolina Experiment Stations where formal cooperative agreements are in effect.

Research on dioscorea propagation has been discontinued owing to completion of essential studies on vegetative propagation. Botanical investigations of plants for annual pulps and on relationships of certain oil-bearing composites have defined the important taxonomic groups of highest interest and will therefore be closed out. Specific research on the collection of plants for insecticidal screening is being reduced and will be undertaken as a phase of the general collecting program for new industrial crops.

The Federal scientific effort devoted to research in New Crops totals 39.7 man-years. Of this number 2.8 are devoted to plant exchange activities, 4.8 to world plant resources research, 4.6 to plant procurement for special activities. Research on new crop evaluation includes 8.3 devoted to evaluation of horticultural crops, 3.8 to agronomic crops, 6.9 to advanced evaluation of potential new crops, 4.0 to the pathological screening of breeding stocks, and 3.5 to maintenance of germ plasm. One man-year is devoted to program leadership.

PROGRESS

A. Plant Introduction

1. Breeding Stock Introduction. The plant exchange program during the past year resulted in 7,747 introductions from and 1,620 shipments to some 100 countries owing to an increasing number of requests by plant scientists for the exchange of breeding stocks. Major introduction emphasis has been on forage legumes and grasses (2,068 items), vegetables (1,916 items), cereals and sorghums (1,679 items).

a. Foreign exchange. Exchange through correspondence provided a number of large collections including 928 forages from Australia, 480 triticums from New Zealand, and 695 tuber-bearing solanums from Wageningen, Netherlands. Exchange with the USSR and satellites accounted for 396 introductions, including a large group of alliums, as compared to 442 items sent to the USSR sphere. Other results of this exchange are a large collection of *Phaseolus* from Chile and 300 capsicums from Latin America.

b. Foreign exploration. Only two foreign explorations were undertaken for breeding stocks. An exploration to Nepal, as part of the ARS/Longwood Gardens cooperative ornamental program, resulted in 230 collections of ornamental trees, shrubs, and herbaceous plants, many of which were introduced to the U. S. for the first time. An expedition undertaken to Mexico in cooperation with the Federal Experiment Station, Mayaguez, P. R., yielded 3,000 cuttings of wild and local garden types of vanilla. This represents the most extensive vanilla germ plasm collection yet obtained to evaluate for improving this crop for Puerto Rico.

c. Domestic exploration. Collecting in cooperation with the four regional new crops programs resulted in the following: NE-9, the collecting of local types of ornamental gourds for genetic evaluation was completed and some collecting of wild blueberries and junipers initiated; NC-7, the completion of a 3-year exploration program for small fruits in Alaska added 135 new collections for evaluation by breeders in the North Central States; S-9, 142 local

fruit and nut varieties from the Gulf States were added to the holding stock of germ plasm at LSU that represents types of unusual hardness, quality, or similar characteristics; W-6, a collecting trip in Oregon and Washington yielded selections of native Ceanothus for trial for erosion, roadside cover, and landscape purposes.

d. Support for AID missions abroad. The special AID plant program to provide research stocks shipped 1,427 lots of seeds and plants to AID missions in 43 countries. In particular, results with sorghums in Thailand to aid development of a new crop, soybeans to Panama for hay and oil production, and corns and barley stocks to improve these crops in Korea show that this program is highly beneficial in efforts to raise living standards in under-developed countries. In the cacao, coffee, and rubber germ plasm program, 225 lots of budwood and/or seedlings were sent to 13 countries and 117 lots received from 10 countries for inclusion in the Miami, Fla., and Glenn Dale, Md., collections. As a result of virus indexing techniques, the Miami cacao collection is comparatively free of known viruses and budwood is moving to foreign countries and to the genetic stock garden at Mayaguez, P. R.

2. Plant Resources

a. Plant identification and classification. Some progress has been made on the taxonomy of Vernonia suggesting that V. anthelmintica may have been introduced to India from Africa and that the bulk of species related to this new oilseed are to be found in Africa. Research on Lolium (ryegrass) has increased knowledge of the geographic distribution of loliums and revealed a little-known species from the Canary Islands. Investigations on Coffea arabica suggest that the species, not previously collected in a wild state, may be indigenous to southwestern Ethiopia. It is important in the study of coffee rust resistance to emphasize the collecting of local types from Ethiopia. Taxonomic studies on Agave resulted in a provisional sectional classification of the subgenus Littea on the basis of floral morphology. A total of 1,162 plant specimens were identified and 389 CR manuscripts were checked for nomenclatorial accuracy.

b. Botanical investigations on new crops. Field study and collecting of Limnanthes in California and Oregon yielded seed of all 8 species and 10 varieties as well as providing field observations to elucidate the growth pattern, ecological adaptation, and other factors essential to selection for further agronomic research. Subsequent chemical analysis showed that oil composition and amount are constant throughout the genus, affording a choice of species for agronomic evaluation.

More than 1,500 seed and plant samples were procured for the oilseed and pulp screening program through our own collectors and P.L. 480 projects. Pulping and botanical evaluations have now shown the concentration of promising pulp species to be in the Gramineae, Leguminosae, Malvaceae, and Euphorbiaceae. From general screening of samples this year, 4 new oilseed species, a new protein source, and a seed gum are highly promising in a group of 40 species which will now undergo preliminary evaluation at Federal and Regional Plant Introduction Stations.

A total of 3,431 plant samples was supplied for extraction in the anti-tumor screening program in cooperation with NIH. Thirty-nine new confirmed-actives were reported bringing the total of active species to 68, representing more than 35 plant families.

Studies on new sources of gums and resins resulted in 5 technical papers pertaining to the world distribution, yield, and propagation of these highly important natural products.

c. P.L. 480 projects. Ten projects are formally active in 9 countries and provided 1,076 collections. These largely entered the new crops screening program but 226 lots of seeds including 93 of Citrullus and 50 of Lactuca were divided and will be evaluated as crop germ plasm. All except 3 recently activated projects have provided seed samples as follows: Turkey - 270; Israel - 180; Spain - 150; Yugoslavia - 266; Pakistan - 170; Uruguay - 40. Forty-two samples of Solanaceae were collected under the Colombian project and are being analyzed for steroid activity.

B. New Crop Evaluation

1. Evaluation of Breeding Stocks. Research emphasis is directed toward locating new sources of disease resistance, determining characters which might enhance adaptation and crop versatility and developing new varieties of agronomic, horticultural, and chemurgic crops, and building-up our germ plasm banks either as seed or vegetative stocks.

a. Fruits and nuts. During the year, 487 new fruit and nut introductions were received, the majority of which required a quarantine period at Glenn Dale, Md. Seventy-four introductions were offered to Federal and State experiment stations. Virus indexing in quarantine remains the governing factor in the distribution of introduced stone fruits. This year, 39 introductions were included in the indexing program and 16 varieties were released as virus negative from the Glenn Dale, Md., Plant Introduction Station.

Research at Chico, Calif., on Actinidia chinensis (Chinese gooseberry) is directed to seed germination, fertility, crop yield, and ripening. Preliminary studies show that alternating cold and warm temperatures during stratification may increase germination, and that for the one variety grown in sufficient quantity, heavy crops are produced in alternate years but does not imply distinct alternate bearing, as is customary for apples. Despite intensive soil fumigation, losses in establishing actinidia seedlings reach about 30% and indicate the need for research on improved soil fumigation techniques in relation to this crop.

Emphasis with stone fruits such as almond, apricot, cherry, peach, and plum at Chico, Calif., is on evaluation of seedling progenies of desirable introductions and the compilation of descriptive catalogs of introductions. For example, seedling populations of the early ripening plum, P.I. 94232 (Persia), and the heavy-bearing plum-cot, P.I. 117682 (USSR) 'Black Alexander', are now in nursery blocks for observation for outstanding fruiting characteristics. Descriptions pertaining to all plum introductions in the Chico collection have been completed and will be distributed to interested researchers.

As a result of detailed observations on 134 apple introductions, a descriptive catalog of early ripening apples has been prepared at Glenn Dale, Md. One such variety, P.I. 143973 (Manitoba, Canada) 'Red Apple', ripens as early as July 30 and is of high quality. The bright red skin color imparts an attractive pink color to the fruit sauce. This apple was discarded in Western Canada since it was not suited to the climate but appears desirable under our conditions and several experiment stations have expressed interest.

Seedlings of P.I. 182831 (Maryland), *Malus* 'Niewland', have been uniformly dwarf at Glenn Dale as compared to progenies of 9 other parents. This suggests the possibility of developing dwarfing stocks which might not transmit viruses owing to their seedling origin, as opposed to vegetatively produced dwarfing stocks. Influence of this seedling line on growth and vigor of scion varieties must yet be determined.

Observations of leaf-spot resistant seedlings of cherry introductions, P.I. 186943 and 202119 (originally from Germany under nos. 127413 and 132028), showed that the resistance to artificial inoculation in 1961 was repeated in the field in 1962 when the plants were subjected to natural infection. Limited progenies of P.I. 202119 showed no natural infection while a population of 218 seedlings of P.I. 186943 had a low incidence of natural leaf-spot infection.

At Miami, evaluations of avocado introductions have shown that the 'Arue', P.I. 99805 (Tahiti), and 'Capac', P.I. 53895 (Ecuador) are

consistently early ripeners as compared to commercial Florida varieties. Seedling populations of these varieties are now in field blocks for observation for earliness combined with heavy bearing and quality. These latter characters are lacking in 'Arue' and 'Capac'.

Cold hardiness and selection of heavy-bearing varieties of lychee are objectives of testing seedling populations at Miami, Fla. Some 1,200 seedlings, marcotts, and grafts were transplanted to the field and were subjected to severely low temperatures this winter. A low of 31°F resulted in cold injury that ranged from minor to extensive leaf and wood killing. Mulching studies initiated this year involving the additional effect of applications of sulphur have shown no immediate treatment effects on seedling growth.

Preliminary evaluation of seedling progenies of subtropical fruits such as Papaya, Malpighia, Myrciaria, and Passiflora show that there is considerable merit to the screening of seedling populations to develop varieties superior in adaptation to those introduced directly from the tropics. A yellow-fruited form of Passiflora (Passion Fruit) has proven resistant to fusarium wilt. Research on fruit set of Passiflora has shown that several factors of incompatibility cause failure of fruit set as some cross combinations result in high fruit set while others produce no results.

Although the regional cooperative programs are not engaged in extensive evaluation of fruits and nuts, the Geneva Introduction Station reported that a strawberry introduction, 'Senga Sengana', P.I. 264680 (Germany), is highly productive with large fruits which maintain size throughout the season. It ripens in mid-season and appears most promising in the northern fringe of the producing areas.

b. Vegetables. In 1962, 1,916 vegetable introductions were distributed to Federal and State research workers, largely through the regional plant introduction stations. One hundred and forty-four potato introductions were indexed at Glenn Dale for viruses and of these 72 were found to be infected with one or more serious virus diseases while 54 were released as virus free or contained only virus S. Eighteen introductions were destroyed as hazardous.

Waterchestnut research at Savannah showed that nitrogen is the key nutrient in production. Increase in production is significantly correlated with rate of N. At an experimental rate equal to 200 lbs/acre of N, the yield was equivalent to 22,550 lbs. of corms/acre. At least one commercial company has attempted trial plantings of waterchestnut with excellent growth and yield.

Under the regional cooperative programs, plant introductions were used in the development of several new vegetable varieties as follows: A new tomato variety 'Marion' released by the South Carolina AES owes

resistance to fusarium and stemphyllium wilt to Lycopersicon pimpinellifolium, P.I. 79532 (Peru). (S-9). The 'Porte' tomato released by CR has inherited similar resistance from this same introduction and the Florida AES developed 'Floralou' with P.I. 79532 and P.I. 126445 (Peru) (L. hirsutum) as sources of multiple disease resistance. (S-9). 'Epoc', a tomato released by Purdue University also owes its disease resistance to P.I. 79532. (NC-7). The Oregon AES has developed a tomato 'German Cherry' selected from P.I. 180725 (Germany). (W-6). Idaho AES developed a new pepper variety 'Idabelle' of which a single plant selection of P.I. 206949 (Turkey) was one parent. (W-6).

In melon varietal improvement, 'Floridew', released by the Florida AES, is considerably resistant to powdery and downy mildew through its parent, P.I. 223637 (Iran). (S-9). In the case of gummy stem blight, however, screening of commercial varieties and plant introductions showed that 4 out of 10 commercial varieties had a satisfactory level of resistance and were as resistant as any foreign introductions tested. (S-9).

Plant introductions were also screened as to their desirability as basic breeding stocks and several were reported this year as follows: P.I. 246502 (Peru) Solanum pennellii, is resistant to several important tomato diseases and has been crossed with Lycopersicon. (W-6). Two accessions of Cucurbita pepo, P.I. 135394 (Afghanistan) and P.I. 172870 (Turkey) are highly tolerant to mosaic virus (S-9) and 10 selections of 5 eggplant introductions (P.I. 286099-108 [Turkey]) out of 300 screened at Geneva have shown consistent levels of resistance to verticillium wilt and are being distributed to breeders. (NE-9). From 500 introductions of okra, Texas AES has selected 7 as having desirable phenotypes for genetic stocks. (S-9). A cucumber introduction, P.I. 179676 (India), is partially resistant to angular leaf spot and powdery mildew. In Wisconsin this trait was transmitted to lines SMR 15 and 18. (NC-7).

c. Field crops. Of about 100 native and exotic economic grasses, about 25 are included in active research programs and some 45 legumes are the subject of state, regional, or national research. Many introductions serve as sources of germ plasm containing desirable agronomic characteristics worthy of transfer to field crop varieties.

Orchardgrass introductions which serve as examples of valuable germ plasm include: P.I. 220877 (Ireland), 230116-117 (Iran), 231551 (Italy), 235474 (Switzerland), and 237265 (Denmark). The accessions of Cynodon dactylon, P.I. 206657 (Turkey), 211021 (Afghanistan), 213384 (S. Africa), 213385 (S. Africa), 213387 (S. Africa), 213389 (S. Africa), 222789 (Iran), and 224691 (S. Africa) show promise of direct usage as pasture grasses or in breeding programs in Arizona

and California. Accessions of Echinochloa, P.I. 183332 (India), 196291 (India), and 223254 (Afghanistan), are reported as being good sources of wildlife food. Germ plasm containing earliness in corn is represented by P.I. 228173 (USSR) and that containing the tillering character is found in P.I. 167959 (Turkey). Two Digitaria accessions, P.I. 279651-652 (Taiwan), were introduced specifically in the search for disease resistance, virus stunting disease, and cold tolerance. A bermudagrass, P.I. 224693 (S. Africa), has entered commercial channels in Texas and is in widespread usage throughout the south-central portion of the state. Exceptional cold hardiness is exhibited by one introduction of blue panicgrass, P.I. 268410 (Afghanistan). This introduction survived the 1961-62 winter at Ames, Iowa, with very little winter injury.

The following varieties of pasture grasses with plant introduction parentages were released during the interim of this report: A new synthetic variety of brome grass, 'Sac', has good resistance to seedling and foliage diseases. The variety was released jointly by the University of Wisconsin and the USDA; it has good vigor and seed producing ability. 'Oahe' wheatgrass, released in South Dakota, is vigorous, early maturing and a good seed producer. A new orchardgrass 'Sterling', an Iowa release, rates high in winter hardiness and in ability to produce excellent stands.

Among the legumes, clovers including white, red, crimson, sweet, persian, alsike, and zig-zag are the subject of much basic and applied research. Introductions of red clover, Trifolium repens, and white clover, T. pratense, are receiving most attention among plant breeders and agronomists throughout the northeastern and central United States. The improvement of other clover species through plant introductions is given in a few reports from widely scattered areas throughout the country. Red and white clovers are being evaluated for winter hardiness and disease resistance. Red clover introductions reported tolerant to powdery mildew and which exhibit winter hardiness include: 228365 (Iran), 234838 (Germany), 234957 (France), 235855 (Sweden), and 260250 (Germany).

Concentration on plant type and root-knot nematode resistance in white clover improvement is reported. A new variety of persian clover 'Gulf Persian' was released jointly by Texas A&M College and USDA. Examples of zig-zag clover, T. medium, introductions reported as being particularly outstanding in vigor, production, and disease resistance include P.I. 284621 (New Zealand), and P.I. 250989 and 253200 (Yugoslavia). Arrowleaf clover, T. vesiculosum, introductions are continuing to be evaluated in southeastern United States with variable results. One introduction, P.I. 233816 (Italy), is outstanding in seedling vigor and yield. A new variety of arrowleaf clover 'Amclo', recently released in Georgia, was derived from

P.I. 234310 (Italy). The new crimson clover 'Frontier' recently jointly released by Mississippi Experiment Station and USDA was derived from P.I. 233812 (Italy). Soil Conservation Service reports from adaptation studies that six clover accessions survived -40°F temperature in Idaho tests; they include three white clovers; P.I. 224680 (England), 231786 (New Hampshire), 234678 (France); two red clovers; P.I. 234941 (Switzerland), 239700 (Switzerland); and one alsike; P.I. 257273 (Sweden).

Reports on the outstanding performance of oilseed crop accessions are as follows: A different type of safflower rust resistance has been found in a single selection from P.I. 253914 (Afghanistan); this line is being used in breeding resistant varieties. A sesame selection from P.I. 170733 (Turkey) produced significantly higher yields than other strains in variety trials in Florida. Two sesame introductions are being used in plant breeding programs to improve the crop. The gene for large capsule size is carried by P.I. 158045 (China) while bacterial leaf-spot resistance is furnished by P.I. 251702 (Yugoslavia). A peanut accession, P.I. 280688 (Mexico), has a deep purple testa which is proving useful as a marker gene in genetic studies. Two new flax varieties, 'Windom' and 'Marine 62', were released jointly by Minnesota and USDA.

d. Ornamentals. The majority of new ornamental introductions have resulted from a series of explorations sponsored by a cooperative program of ARS and Longwood Gardens, Kennett Square, Pennsylvania. Evaluations of these and other introduced ornamentals by cooperators of state experiment stations and similar institutions form the basis of recommendations for releases to the nursery trade.

The Montana Agricultural Experiment Station's research workers have selected six of the 1956 Japanese chrysanthemum introductions as parents for use in breeding chrysanthemum strains with florist-type flowers. A seedling ('Susan Leight' X P.I. 235927) will be released to the trade in the near future. Four summer-flowering, virus-free Japanese chrysanthemums under test at Bozeman, Montana, show promise as early garden varieties sufficiently winter hardy to withstand climatic conditions in that area. 'Kinkazan' (P.I. 231099) is a profusely flowering, deep yellow pompon type with vigorous growth and strong stems; 'Hinomaru' (P.I. 231096) is an attractive lavender-pink pompon, well-liked type except for its tendency to produce weak flower stems; 'Shinmison' (P.I. 231100) is a lavender purple pompon type of good quality with strong stems; and 'Tsukase' (P.I. 231102) is a white double-flowered type with erect growth habit, 20 inches tall, and stiff flower stems. 'Kinkazan' is well liked in North Carolina, Oklahoma, Texas, Virginia; 'Hinomaru' is liked in Louisiana, North Carolina, and South Carolina. (W-6). After four years of testing at the North Platte Experiment Station, the

Nebraska Agricultural Experiment Station released to the trade the Japanese chrysanthemum varieties; 'Virgin Elegance' (P.I. 235624) a deep purplish-red, heavy blooming, upright, stiff-stemmed variety 30 inches in height and outstanding for flower-color retention even at high temperatures; 'Blue Sky', (P.I. 235627), which bears pure white flowers 2-1/2 to 3 inches in diameter, dense clusters suitable for cutting. 'Blue Sky' is unusually frost tolerant for a white variety. Both varieties are cold hardy and early enough to be dependable in their flowering. (NC-7).

Two introductions of *Eurya*, P.I. 235425 (Japan) (*E. emarginata*) and P.I. 240914 (Japan) (*E. emarginata* var. *microphylla*), distributed by the Glenn Dale Plant Introduction Station in recent years, have received favorable comment by cooperators in the southeastern states who indicate that given protection these introductions show considerable promise as semi-dwarf evergreens for rock gardens and where low shrubs are required.

The Proteaceae of South Africa contain many handsome shrubs which bear large attractive flowers. Seedlings of seven 1961 introductions are under evaluation at the U. S. Plant Introduction Station, Chico, Calif. Three introductions, *Leucadendron galpinii* (P.I. 273807), *L. dregei* (P.I. 273806), and *L. sp.* (P.I. 273809), withstood 15°F in January 1963 with relatively little injury. *L. dregei* bears attractive large pink cones, has dark green foliage, and has a low, spreading shrub-like growth habit which makes it suitable for foundation planting. *L. galpinii* is an interesting conal fruited novelty but not as handsome as *L. dregei*. Evaluation of this seedling collection will be continued for several years before planning a wider test throughout the southwest of those considered most promising.

Of 27 seedlings of *Dombeya elegans* planted at the Miami Plant Introduction Station in October 1961, 16 flowered this season exhibiting considerable variation for so small a population. Flower color on most plants was identical to the parent plant, but two plants bore flowers of a dark rose which was even more outstanding than the pink color of the parent. Although cuttings are difficult to root, a successful method has now been developed. This plant produces large clusters of flowers on the periphery of the plant; it holds much promise as a pot plant for the florist trade or as a small ornamental tree.

2. Maintenance of germ plasm

An additional 5,000 lots of seed have been deposited in the National Seed Storage Laboratory, bringing the total to over 22,000 collections. The largest groups are composed of world collections of rice and flax.

Germination tests have been run on some lots of seed in storage for 2, 3, or 4 years, and no loss in viability has been evident thus far.

After 4 years of storage there appear to be seeds of three varieties of peas, two of beans, two of corn, and two of watermelon which apparently are maintaining their viability better under adverse conditions than the other varieties in storage. The apparent differences, however, will have to be confirmed by seeds obtained from other crop years' production. So far as storage conditions are concerned, 70° with 50% R.H. appears to be only slightly more favorable than 70° and 70% R.H. Seeds stored at 90° and 50% R.H. are deteriorating more rapidly than those stored at 50° and 90% R.H.

Seeds of lettuce, safflower, sesame, crimson clover, and sorghum stored with 4% moisture in various gases had negligible losses in viability over a 24-month period. However, when moisture content was increased to 7% and 10%, sensitivity to temperature increased and viability varied according to crop species. At the end of the second year, sorghum seeds stored in partial vacuum germinated significantly higher than seeds held under various other atmospheres in sealed metal cans. There were no consistent differences in results due to atmospheres for any of the other kinds of seeds used.

The survey of fruit and nut clones in the U. S. requested by the National Coordinating Committee for New Crops is being prepared in 3 parts. The first part which deals with apples consists of approximately 5,509 entries. It was completed and will be available for distribution in 1963. Part 2 of the survey dealing with stone fruits is two-thirds completed. Approximately 5,848 items are covered in this part. The 3rd part of the survey covers pears, nuts, and miscellaneous fruits such as small fruits, subtropical fruits, etc. This part consists of about 5,565 items.

3. Chemurgic Crops

a. Oilseeds. Crambe abyssinica, a potential source of erucic acid in the seed oil, was successfully produced under dry land (up to 1100 lbs/A) and irrigated (up to 2800 lbs/A) conditions in the northwestern United States. Fertilizer studies indicated that relatively low rates of nitrogen (similar to barley) will be required. Spacing experiments indicated that the crop will be most productive when drilled in rows 6 to 14 inches apart at seeding rates of 5 to 10 pounds per acre. It was established that planting should be as early as possible for spring plantings in the northwestern states. Arrangements were made with growers in Oregon, Washington, Montana, and Wyoming to produce approximately 100,000 pounds of seed (in pod) in 1963 for extraction and distribution of oil to interested industries for use in product evaluation.

Vernonia anthelmintica, a highly promising source of epoxy fatty acid in the seed oil, produced yields above 1000 pounds per acre in experimental plots in Nebraska.

Limnanthes, a potential source of long chain fatty acids in the seed oil, was planted in the fall, survived winter temperatures at Glenn Dale, Md., and produced seed in early May. Observations of morphologic change when plants were covered with snow indicated that growth actually took place during this period.

b. Annual pulp fiber crops. As potential sources of paper pulp, kenaf (Hibiscus cannabinus), Crotalaria juncea, and Sorghum alnum are considered promising for crop development. Dry-matter yields in 1961 and 1962 were, in general, superior to those of previous years.

In the investigations of quantitative morphologic development of new crop species such as kenaf and Crotalaria, precise methods were developed for measuring the influence of environmental factors on plant growth. Morphologic methods of identifying plant responses to environment should prove to be a valuable tool not only for new chemurgic crops but also for established crops.

c. Other new crops. Growth of Dioscorea spiculiflora was found to be governed rather closely by special environmental conditions. At least three weeks of continuous high temperature above 80°F is necessary for sprout initiation on tubers and at least two more weeks at approximately 90°F for sprout elongation. This period should be followed by long days, high temperatures, and plentiful moisture for rapid vegetative growth. In the fall, short days (12 hours or less) induce flowering and subsequent senescence of the plants. The tubers then become dormant and require a rest period of 2 to 3 months.

Bamboo, a potential source of paper pulp and a replacement crop for the South, is being established in large-scale experimental plots. The planting at Camden, Ala., is nearing completion and will be used for comparisons of harvesting cycles, cultural practices, harvesting methods and in comparison with pine and poplar pulp sources.

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TROPICAL AND SUBARCTIC RESEARCH PROGRAMS
Crops Research Division, ARS

Problem. These programs are concerned with agricultural research problems existing under the extreme environments of both the tropics and the subarctic. Use is also made of these climatic extremes to complement, expand and accelerate coordinated research programs conducted in the temperate zone of the continental United States.

Program headquarters are located in Alaska, Puerto Rico and the Virgin Islands, and are under or coordinated by the Crops Research Division. The work is organized to broaden our knowledge of agriculture and strengthen the industry locally. Opportunities also exist to extend the scope of basic research in the United States by utilizing both natural tropical and subarctic environments.

The current department program at the Alaska Agricultural Experiment Station is based on Congressional action in 1949 providing for joint responsibility and administration by the University of Alaska and the Agricultural Research Service of USDA. Direct Federal funds have been appropriated annually since 1949 which, under a Memorandum of Understanding, provide for direct Federal support in addition to the Federal grant funds (Cooperative State Experiment Station Service) available to the Experiment Station. Directly appropriated funds are provided to accelerate and strengthen the agricultural status of Alaska, to improve the State's self-sufficiency in food and forage, to better its civil defense posture, and to foster family farm units.

Research opportunities are twofold in Alaska. One is that of utilizing the unique environment of a subarctic location to contribute to our basic knowledge of agriculture. The other is to further research findings contributing to the economic well-being of Alaska's agriculture and its self-sufficiency in a strategically important location on the North American continent.

The Federal Experiment Station in Puerto Rico (FESPR) was established in 1901 and serves as the tropical research center of the Department. It conducts a program of basic and applied research on tropical plants, especially those of importance to mainland economy. It also serves as a base of operations and provides facilities for winter-testing and seed-increase projects. These permit growing two crops a year (one on the mainland during the summer and a second in Puerto Rico during the winter) and greatly accelerate temperate-zone breeding and testing programs. The station also serves the United States as a source and experimental center for new- and replacement-crop materials from the tropics. In addition to Crops Research activities, the Entomology Research Division, Plant Quarantine, and the Soil

Conservation Service also have operations based at the Federal Experiment Station in Mayaguez.

The Virgin Islands Agricultural Research and Extension Program (VIAP) was initiated to give greater economic stability to the agriculture and people of these Islands under unique sociological, environmental, and economic conditions. Low and irregular rainfall, undeveloped markets, difficult communications, undependable transportation, and ignorance of modern farming methods complicate the problem. Education through demonstrational research and extension activities is the best approach to raise the standards of the people concerned.

PROGRAM

The Department conducts three diverse programs in Palmer and College, Alaska, at Mayaguez, Puerto Rico, and on St. Croix in the Virgin Islands, each distinct and fitted to the needs and facilities available at these locations. In Alaska the basic and applied research disciplines comprise soil and water utilization, crops research, plant diseases, entomology, marketing, management, economics, machinery, structures, material handling, and animal husbandry. Cooperation is effected with the Alaska Crop Reporting Service, the Alaska Division of Agriculture, Alaska's Cooperative Extension Service, the Soil Conservation Service, Matanuska Maid, Incorporated (the local farm cooperative) and other food wholesalers and retailers, U. S. Geological Survey, U. S. Weather Bureau, the North Central Region (of the experiment station's organization), various Canadian Experiment Stations, and the Matanuska Valley Breeders Association.

In Puerto Rico, the basic and applied program is devoted to both temperate- and tropical-zone crops, utilizing the tropical environment to broaden and expedite research findings. The work comprises investigations with insecticidal, drug, food, spice and special, and forage crops, covering also plant introduction and testing, weed control, plant disease, and winter-testing and seed-increase activities. The program is closely cooperative with the Commonwealth of Puerto Rico. Those phases dealing particularly with the Island's crops are cooperative with the Agricultural Experiment Station of the University of Puerto Rico. The research on blood agglutinins is cooperative with Boston University, Boston, Mass., that on Cacao with the American Cocoa Research Institute, Washington, D. C., that on Vanilla with the Flavoring Extract Manufacturers' Association, and the Vanilla Bean Association, and that on pepper with McCormick & Co., Baltimore, Md. The research on Tephrosia and Dioscorea is cooperative with the New Crops Research Branch and that on cereals with the Cereal Crops Research Branch, both of ARS.

The Virgin Islands Program is small, being devoted mostly to demonstrational types of research and extension service in the fields of soil science, animal husbandry, plant science and home economics.

Cooperation is effected with the Virgin Islands Corporation of the Department of Interior.

The Federal scientific effort devoted to the programs of tropical and subarctic research totals 23.2 professional man-years, including one for program leadership. Of this number 9.2 are devoted to subarctic research: in soil and water utilization 1.2; crops research 3.8; plant diseases 0.5; entomology 0.5; marketing, management, and economics 1.0; machinery, structures and material handling 0.8; and animal husbandry 1.4. The Federal and Federal-grant/State research are combined into a completely integrated program. The latter portion of this program was reported in 1962 to comprise 9.4 professional man-years as follows: soil and water utilization 1.5; crops research 3.4; plant diseases 0.5; entomology 0.5; marketing, management and economics 2.0; machinery, structures and material handling 0.8; and animal husbandry 0.9.

The tropical program in Puerto Rico entails a total of 8 professional man-years as follows: insecticidal crops 1.0; drug crops 2.5; food crops 0.5; plant introduction and testing 1.0; spice and special crops 1.5; plant diseases 0.3; winter testing and seed increase 1.2.

The Virgin Islands total is 5 professional man-years comprising soil science 0.5; animal husbandry 1.5; plant science 2.0; and home economics 1.0.

PROGRESS

A. Alaska (Integrated total program)

1. Soil and Water Utilization. Water intake (measured with a Purdue sprinkling infiltrometer) of several Matanuska Valley soils was found similar to that exhibited by Mississippi loessial materials. Greater differences were attributed to surface characteristics than to profiles. For example, old pastured turf accepted precipitation at only 50 per cent of the rate of an adjoining tilled plot. Water intake rates of Bodenburg silt loam were found to differ 14 per cent between seasons. The most widely cultivated soil series in Alaska's Matanuska Valley display relatively rapid intake rates, especially when their surfaces are tilled.

Recent homesteading activity has been greatest in podzolized soil areas extending west from the Matanuska Valley into the relatively unknown Susitna basin. Here lime, boron and all major nutrients (nitrogen, phosphate, potash) are deficient for general cropping and gardening. Greenhouse and field plot trials showed that most crop plants respond markedly to these amendments although the winter survival of perennial legumes was reduced by high fertilizer rates. Urea again proved inferior to ammonium nitrate as a midsummer top dressing for grass. Of new fertilizer materials and formulations,

highly water soluble mixtures proved more effective than slowly soluble mixtures; calcium metaphosphate (a new TVA compound) was as effective as treblesuperphosphate in improving yields on Bodenbug silt loam. Diammonium phosphate was evaluated. Calcium silicate in greenhouse trials was an inferior source of calcium in podzols containing abundant allophane.

Potassium requirements of intensively managed forage grasses are considerably greater than expected a decade ago. In South Central Alaska large K applications increased both brome grass and timothy yields, nitrogen recovery and uptake both being stimulated. The ratio between calcium and magnesium in Alaskan potato foliage and tubers seems low compared to the ratio found in tubers grown in other states. Potassium nitrate is surprisingly inferior to K_2SO_4 and KCl as a source of K for romaine lettuce.

Evaluation of Alaska's land capability potential will continue through cooperation with the U. S. Forest Service, the Soil Conservation Service and Alaska's Department of Natural Resources, although a separate line project has been terminated. Soil surveying responsibility has been assumed by the State Division of Lands and SCS. Priorities and legends are established through consultation with the Experiment Station staff and other rural agencies.

A small acreage cleared at College in 1957 required five years before sufficient internal drainage developed through gradual permafrost recession to permit cropping with modern machinery. A windrow draft machine and a mechanical root picker were further evaluated by cooperating farmers. Efficient utilization of cleared land requires supplemental irrigation. Additional water during normally dry spring weather again facilitated the emergence of small seeded annual forages, aided in reducing weed competition by fostering rapid response to selective herbicides, and insured larger, earlier yields. Annual ryegrass exhibited large forage potentials when its early growth was stimulated by irrigation and weed control; annual forages may be significant in reducing late utilization pressures on perennial brome and timothy stands.

2. Crops

a. Agronomic. Continual winterkill losses of perennial forages and turf in the Matanuska Valley emphasize a need for improved varieties and for more knowledge of local micro-environments and their relationship to fall hardening, winter survival, and spring growth initiation. The vast gene pool of native Alaska flora is being utilized in grass breeding programs.

A new synthetic brome grass strain (Alaska B-1) again displayed superior hardiness during the 1961-62 winter, emerging little damaged after severe conditions completely killed adjoining plots

of Manchur, Canadian Commercial and Carleton; 103 pounds of seed were obtained from this new strain that also possesses superior agronomic characteristics such as lodging resistance and leafiness. Four additional new synthetic brome strains were assembled -- three combining a good agronomic performance with high, low and intermediate fertility, the other combining performance plus selected plant type features. One objective is to relate fertility of synthetic strains to that of their parents.

Cytological analysis of brome parental materials at hand was begun. Among the accessions a 28 chromosome tetraploid brome grass was discovered, having been collected from the lower Yukon Valley. The northernmost extension of this particular species was formerly thought to lie some 700 miles south of that area.

Eight new synthetic timothy lines have been assembled for field evaluation. Stocks of seed from polycrossed alfalfas were increased in number and quantity. Sufficient seed of a superior line of alfalfa (Medicago sativa crosses) to undertake field trials was obtained by bee pollination in a greenhouse. Over 300 red clover lines were established in the field in progeny rows for further agronomic evaluation. Foundation seed stock of Alaskland red clover was increased. Agroelymus C₃ generation progenies displayed considerable interspecific recombinations.

Artificial shortening of the day length during the onset of cold weather fostered cold resistance development in field planted alfalfa, as contrasted to plants exposed to normal and prolonged day length. At the College Farm a three-year response of alfalfa to annual spring topdressings of 200 pounds of K₂O per acre nearly doubled dry matter production over yields obtained where only 50 pounds of K₂O was applied.

The crude protein content of cereal grains appears little related to variety. Fertility levels and other environmental factors, rather than genetic inheritance, account for major differences in crude protein content of grain. When grown for roughage, early maturing cereal varieties tend to contain more protein while late varieties tend toward greater gross yields. No statistical or economic significance was, however, discovered in the yield and protein content of late and early varieties.

Of some 80 lines of x-ray and neutron treated wheat, oats and barley (originally selected because of earlier maturity or improved lodging resistance), no outstanding materials were seen in single plot yield trials. A nursery of 2100 progeny rows derived from ethylenimine treated seed (1960) displayed little variability although several hundred individual cereal plants were selected for further observation. Genetically pure seed stocks were obtained for agronomic performance comparisons on a pilot basis. In general, this 8-year

old program seeking better adapted cereals through induced mutation has not been as productive as originally hoped. More rapid progress is promised by traditional breeding methods.

Over half of the cereal varieties and strains tested at Palmer and Fairbanks in 1,000 yield test plots were selections from the breeding program, indicating a shift from past emphasis on evaluating introduced varieties. Much of the breeding material is in the preliminary testing stage. Several hundred additional selections were made during the year. Preliminary evaluation of yield and performance for several years indicates that variety-location interactions between Palmer and Fairbanks are important in yield, maturity and lodging measurements. Current advanced yield tests are programmed for further determination of interaction effects.

Following the suggestions of a comprehensive review committee, breeding emphasis has been focused on barley. A series of 16 lines are currently being combined into a multiple hybrid. In addition, F₂ populations of three barley hybrids are being produced for detailed study. Minor emphasis is being given oat and wheat hybridization.

Analysis of data from 200 spaced plants of Edda barley revealed a range in yield component performance (tillers per plant 4-29; kernels per head 21-79; and weight per 100 kernels, 4.03 - 5.89 gms). Further research will be necessary to determine whether these within-variety yield component responses are consistent, and if response as spaced plants has any relationship to solid planting yields.

Preliminary evaluation of several herbicides applied pre-emergence indicated that 3,4-dichloropropionanilide may be valuable in oats and barley underseeded with grasses and/or legumes. All crops displayed good tolerance to this chemical at 1 1/2 to 3 pounds per acre. Excellent weed control was obtained. DCMA at 2 to 10 pounds per acre and propazine at 1/2 to 7 pounds per acre applied pre-emergence gave good weed control in oats, barley and timothy but damaged red clover. 2-methoxy-3,6-dichlorobenzoic acid at 1 1/2 to 2 pounds per acre gave total eradication of knotweed, a weed difficult to eradicate in Kentucky bluegrass turf. Also controlled were dandelion, chickweed and shepherds purse. Pineappleweed was controlled at 2 to 2 1/2 pounds per acre but at the higher rate there was a slight indication of injury to bluegrass.

Invasion of perennial grass fields by woody species is a chronic problem. A replicated test for controlling brush encroachments on cleared land with fenuron pellets applied at three rates was started in May. Dominant species were birch and alder. Some leaf curl was seen late in the season but no conclusive killing was noted.

The effectiveness of 31 herbicides was evaluated for 10 horticultural crops, 4 agronomic crops and turfgrass. The use of an exponential sprayer has speeded preliminary evaluation of herbicides. Herbicides displaying potentials for commercial purposes are Alipur and barban (pre-emergence) for sugar beets; Solan, DCMA and propazine (post-transplant) for celery; DCPA, CDEC and ametryne (pre-emergence) for seeded cabbage; ipazine, propazine, Solan (pre-emergence) and ipazine, propazine, linuron and Solan (post-emergence) for carrots; DPA, CIPC and CDEC for lettuce; and 2-(MCP) on established lawns. Recommendations for commercial use await official FDA clearance.

The severe winter of 1961-62 completely eliminated introduced turfgrass plantings, although Alaskan selections were injured only slightly. Increase blocks were seeded of 8 Poa and 8 Festuca selections. Some 160 selections were made from a nursery established in 1960, seed being harvested from these plants. Selections were chosen for vigor and freedom from rust and mildew. About 360 Poa and 130 Festuca accessions were established in spaced-plant nurseries, with Merion bluegrass and Olds red fescue included as checks. Much rust and mildew occurred in the Poas. Ninety lots were free of mildew, 190 free of rust, and 35 free of both. Merion was free of mildew but rusted. Many progenies exhibited segregation for disease reaction and growth type. Fescues showed little disease and extreme variation. Small broadcast plots of 112 selections made in previous years were seeded for preliminary evaluation under turf management. Rapid emergence and high seedling vigor were characteristic of northern accessions. First year performance indicated high turf quality in some. Poa alpina produced thick, rugged turf. Sixteen named turf varieties were seeded in 1962 at Palmer and evaluated under 2 N levels and 2 clipping heights. Six pounds of N per 1,000 square feet appears necessary for good turf. Best appearing bluegrass was Merion and best fescue was an Alaskan selection. Three tests were seeded to relate seed production of Merion bluegrass and Olds red fescue to (1) seedling year management, (2) N levels, (3) post seed harvest management. At College, Thiram was the most effective of several fungicides for controlling snow mold on bluegrass. Fall fertilization predisposes turf to snow mold. An ecotype of arctic and boreal grasses was expanded and reorganized by addition of accessions of Poa, Festuca and Agrostis. The uncontaminated gene system of Bromus pumpellianus specimens collected from the northernmost arctic extremes of its range are an invaluable asset in working out taxonomic relationships in the B. inermispumpellianus complex.

b. Vegetables. Hollowheart in Alaska grown potatoes poses critical marketing problems since the entire crop is sold as tablestock. Studies focused on factors predisposing tubers to hollowheart revealed the presence of chlorogenic acid in internal necrotic spots. Three years of fertilization studies at College and Palmer show that heavy nitrogen fertilization increases the number of hollowheart

tubers without a consistent increase in yields. Under otherwise similar production practices, two inches of supplemental water did not increase hollowheart tubers in either Kennebec or Alaska 114 at Matanuska. An 8-hour day length imposed on field stands of Alaska 114 at College for 21 days after tuber initiation reduced hollowheart incidence.

Because the market season is now extended to 12 months, sprout control of stored potato tablestock is necessary. At College, 3 pounds per acre of MH-30, applied as a foliar spray on August 15, provided effective sprout control. Other effective sprout control agents for tubers held at storage temperatures ranging from 40 to 60°F were CIPC, MENA and Fusarex. A combination of CIPC, a fungicide and wax applied to harvested tubers reduced weight loss, sprouting and rotting of stored tubers. A specific growth inhibition of tuber sprouts induced by light has been observed.

Several promising potato clones possessing frost resistant foliage were further evaluated in the field. One is sufficiently hardy to be valuable as a home garden strain in many parts of Alaska where summer frosts kill back the foliage of standard varieties. A new russet-skinned line was tested by commercial growers who conclude that it offers several advantages and are requesting its official release. Several clonal lines selected from an Ontario x Stately cross yielded excellent tubers for chipping purposes.

Improved cultural practices are needed for truck crop production. Practices being emphasized are attempts to manipulate micro-environments. For example, at College (64° latitude) new plastic mulches in several forms raised mean monthly soil temperatures. Higher soil temperatures enhanced yields of field planted squash and tomatoes. Of the mulches evaluated (clear polyethylene, petroleum and latex emulsions) each possessed distinct advantages and disadvantages. Foliar sprays of sugar and gibberellic acid show promise for repressing or stimulating plant vegetative growth and crop yields. Vegetative growth repressants may prove valuable in inducing maturity and thus encouraging fruit ripening. An improved cabbage selection (61-A) stored six months in saran and trycite wrappings retained its excellent quality with little storage loss.

In the Matanuska Valley Freezer 37 and Midfreezer peas were prime for freezing by August 1 and August 6, respectively.

c. Fruits. Winter environmental conditions at College were further defined by a complete record of root and crown temperatures of strawberry plants grown in an open field, under various mulch protection and snow cover. Crown temperatures of 19° in December were the lowest for the 1962 winter. An extensive Sitka hybrid planting sustained 60 per cent mortality while F. glauca sustained only 15 per cent loss. Mortality was not reduced by straw mulch. Snow coverings of 12 and

28 inches were not reflected in differential survival.

Of regional interest to other states and countries is the largely untapped reservoir of winterhardy plant genetic materials indigenous to Alaska. A small-fruit nursery of collected acquisitions was maintained, some clones being expanded for further evaluation.

3. Plant Disease. Seed treatment and crop rotation may be effective in controlling carrot losses due to Stemphyllium radicum. This disease organism apparently overwinters in Matanuska Valley soils under certain conditions and was found responsive to light when cultured in the laboratory. Colony size is restricted in the absence of light but resumes growth when again exposed to light. The inhibitory substance is ether extractable and thermostable.

A new storage rot not previously reported in Alaska but found infesting celery stalks has been tentatively identified as Thielaviopsis basicola. Engmo timothy seems particularly susceptible to the disease Heterosporium phlei, especially when nutrition is inadequate. During the collection of indigenous legumes, rampant root and crown rots were discovered in widely distributed areas. Although the causal organisms have not yet been identified, the spread of inoculum and its pathogenicity on these wild hosts may pose difficulties in utilizing legumes other than peas in cropping systems. Other significant diseases again recognized were Helminthosporium teres on barley and Rynchosporium on brome grass. Native grasses in nursery culture displayed a wide range of susceptibility to rusts and mildews.

Gains were made in developing techniques of manipulating S. scabies in laboratory cultures, an investigation undertaken to define significant growth factors so that effective controls may be devised. It was facilitated by the availability of sophisticated laboratories at the Mikrobiologisk Institut, Landbrukshögskole, Vollebakk, Norway, where much of the work was done. Growth, sporulation and spore germination were observed to delineate the cause, amount and nature of cultural variability. S. scabies tolerates a wide range of nutrient conditions, some growth taking place on only water agar. Growth on cellophane membranes superimposed on agar media eliminated its hardness as a growth factor. With media surfaces physically equivalent, media pH is much more significant, growth occurring only within a narrow range. Sporulation was influenced by pH, hardness of the agar, light and nutrition. The pH range for sporulation is narrower than for growth, possibly because aerial mycelia must develop before sporulation takes place. The reaction of a buffered agar medium is not necessarily that of the colony micro-environment. Many factors involved in spore germination have not yet been elucidated. Considerable inhibition has been observed, which can be partly overcome by a more alkaline reaction or by modifying other supplemental growth factors. Actinophage is common

in scab postules and may contribute to variability encountered in field trials.

Virus X free potato stocks (Kennebec) were again increased, only one positively reacting clone being found in the 1961 yield. An elite line has been established for further multiplication. Tuber units segregated because of suspected leaf-roll and calico are preserved to develop test sampling techniques.

4. Animal Industry. During the past five years average production of the Matanuska herd (28.3 cows) was 11,534 pounds of milk containing 464 pounds of fat. In contrast 1948-1953 production (16.5 cows) was only 6,495 pounds of milk (290 of fat). A large part of this gain is attributed to up-grading by cross-breeding and infusing Holstein and Red Dane bloodlines into the original Guernsey stock. Eight sister production comparisons have now been accumulated; the average of eight Dane-Holstein crosses (14 records) is 14,444 pounds of milk (537 pounds of fat), while their purebred sisters (17 records) averaged 14,606 pounds of milk (529 pounds of fat); their dams production (25 records) is 13,412 pounds of milk and 494 pounds of fat. An example of the excellent Holstein bloodline available in Alaska is seen in last year's record of Alaska Genlin Clothilde (a registered 6-year old dam) in the Matanuska herd. She produced 19,326 pounds of milk (740 pounds of fat, 1,695 pounds of solids-not-fat) in 305 days (2x). Other best records for the year were 16,300 pounds by a 4-year old dam, 19,060 pounds by a purebred Holstein in the College herd, and 17,610 pounds and 14,860 pounds by two paternal sisters.

Minimum shelter during early growth (as opposed to warm barn housing) does not seem to reduce the later production of good heifers. This conclusion is supported by the performance of 16 heifers raised in a warm barn compared with 15 raised outside under minimum shelter. Each group contained one non-breeder. Four of the minimum sheltered heifers calved late because they were difficult to catch in heat. Average weights at three years were 1205 pounds for those raised "outside" versus 1165 pounds for the "inside" group (Ragsdale normal is 1165 pounds).

Frozen semen conception rates markedly improved during the year. First service conception stands at 57.6 per cent -- only 2.3 per cent below fresh semen rates. Frozen semen is now stored in liquid nitrogen, a more satisfactory holding temperature than afforded by dry ice. Local supplies of liquid nitrogen are not stable because of small volumes manufactured. For this reason total reliance by Alaska's dairy industry on imported frozen semen is not realistic at this time. Although new germ plasm is available through frozen semen, it is unlikely that better producing lines than already at hand in the local stud (which has produced three Honors Lists dams) can be so obtained.

Dairy rations containing meat meal and bone meal manufactured in Anchorage gave slightly lower milk production than a ration containing an imported commercial concentrate. Urea did not improve the meat meal ration. Despite inferior animal response, local meat meal and bone meal give a cheaper concentrate than can be imported, a price advantage offsetting its lower milk production potential.

Loose housing of a dairy herd throughout the subarctic winter experienced at the College Farm (latitude 64°) compares favorably with warm barn quarters in many respects. Judging from the response of six cows managed under each system, better health was attained by those animals living in an open shed. Gaining weight and displaying no lameness or swollen joints, they gave less milk than their counterparts who enjoyed warm quarters. Outside temperatures for the 6-month test period averaged 23°F, one cold 10-day period averaging -51.3°. Temperatures inside the open shed ranged from 10 to 15° warmer. A significant limitation is encountered in feeding silage outdoors because cows cannot ingest enough frozen roughage. Electrical heaters were not satisfactory in keeping silage unfrozen.

Weaning dairy calves 25 days old and thereafter feeding them in groups saves both labor and feed costs. An animal protein ration based on locally available meat meal gave a savings of \$20 a ton over an imported plant protein ration. These findings are from a comparison of groups of calves fed hay after weaning, plus a 4-pound daily offering of a 25 per cent protein concentrate containing either plant or animal protein. While the ration containing meat meal was at first reluctantly accepted, the group on this ration finally consumed it in sufficient quantity to attain good growth.

Veal raised on milk replacer is eagerly accepted by Anchorage consumers, but an economic feasibility study indicates low return for labor and skilled management under present cost and price structures. Superior weight gains were made by calves fed milk replacers (reconstituted to 10 per cent solids) at 12 1/2 per cent of body weight to 30 days and 15 per cent of body weight thereafter. Weaning at greater than 200 pounds liveweight increased costs and decreased consumer acceptance.

5. Marketing, Management, Economics. In response to activity stimulated by national Rural Area Development programs, economic feasibility estimates were prepared for several specific farm enterprises and marketing facilities in the field of meat and poultry. An investigation of fresh milk price versus consumption involving the cooperation of 34 local schools and two dairy firms proved that a downward adjustment from 8¢ to 5¢ per half pint raised consumption from 45 to 153 per cent, depending on school administration attitudes. Only 50 per cent of Alaska's school children are covered by the Special Milk Program even though 90

per cent might be reached. This large population requires 6 million half pints per school year compared to the current consumption of 1.9 million.

Anchorage residents consume 355 fresh eggs a year, eight more than the national average. Analysis of 459 dozen eggs purchased from retail stores revealed that locally produced eggs were generally inferior to imported eggs in uniformity and prevalence of blood spots, package appearance, and other minor characteristics. On the other hand, many consumers could not distinguish these differences, mostly of small magnitude but of high statistical significance. Many consumers favor local eggs because certain brands are usually fresh enough to poach. Quality of both local and imported eggs is better in winter.

Wide differences in the characteristics of homestead farms and commercial farms in railbelt Alaska were defined in an evaluation of agricultural credit needs. Of 93 enterprises studied, 30 were homestead-beginning farmer type with little or no farm income. Significant characteristics of the farms and farmers were: all but one farm were owner-operated, only two farmers had been born in Alaska, only one farm had been acquired within the family. The 30 homesteaders had been in Alaska an average of 12 years, on their farms 4 years. Commercial farmers were 14-year residents with 10 years on their present farms. Homesteader-farmers had average total assets of \$30,000, of which \$2,200 was borrowed. Commercial farms had gross assets of \$99,600 with outstanding debts of \$29,800. Of these commercial ventures, dairy farms with gross assets of \$106,600 and debts of \$42,500 were the largest. Vegetable and potato farms had the largest owner equity (\$95,678), the smallest debt (\$9,058) and the best loan repayment record. Poultry farms had the least gross assets (\$66,000) and the poorest loan repayment record. Average annual net cash income of the commercial farms was \$6,097 (dairying \$7,551, poultry \$1,403). Fifteen dairy farms selected for more detailed analysis had an average herd size of 42 cows, and an annual production per cow of 8,132 pounds of milk. Cash costs to produce a cwt of milk was \$8.05. A five-year summary revealed that dairy farms had doubled in herd size and production. Total investment had gone up 75 per cent and indebtedness 61 per cent. Net income per farm had climbed \$2,950 in five years.

Gaps in Alaska's marketing and price statistics normally filled by other agencies continue as a research responsibility. Among these is outlook information indicating market trends, forecasting demands and opportunities, and devising recommendations concerning the marketing of specific crops and livestock. Interpretation of production statistics compiled by the Alaska Crop Reporting Service and the Alaska Division of Agriculture is a vital need in planning new research approaches and coordinating agency approaches to developmental obstacles.

Valley-wide sampling of grain revealed the mean moisture of barley at harvest to be 24.2 per cent, of oats to be 20.7 per cent. Satisfactory drying of 32 per cent moist grain in a prototype farm bin was accomplished at a cost of 1.89 cents per bushel. Germination of stored grain seed containing 22 per cent moisture again was not reduced by freezing although an incidental invasion of fungi was harmful.

6. Structures and Materials Handling. Alaska's normally wet harvest season often demands emergency techniques for storing relatively wet grain. Fermenting wet feed grain has again been proved a valuable method of cheaply storing a high energy feed. New inexpensive temporary commercial bins and a wider availability of on-farm feed mills may encourage adoption of fermented storage. Alaska's relatively cold storage period permits satisfactory holding from harvest to spring planting of seed grain at 20 per cent moisture.

A summary of 10-year heat losses through various walls of Alaskan timber and insulating materials is now being prepared. Input loss relationships as influenced by interior-exterior temperature differentials, by exterior air velocity, and by leakage will be revealed.

7. Entomology. Summer handling and herding may be facilitated by warble control, thus reducing a pest that is perhaps a major cause of "spooking" reindeer. Most reindeer hides are now valueless because of warble scar damage. Early August treatment of fawns on Nunivak Island (with Ruelene, Tiguvon and Famophos) did not give as effective control as late August treatment. Having defined an effective dosage and a satisfactory administration technique, 1962 studies were transferred to Selawik where privately owned animals were made available for further evaluation of satisfactory management practices. Famophos or Tiguvon was subcutaneously injected into 100 male fawns at 2cc and 3/4cc per hundred pounds of body weight, respectively. Since this herd is rounded up in November, subfreezing temperatures rule out other administration methods.

Contributing to a regional attack on Melanoplus bilaterus bilaterus (grasshopper) is an investigation of abundance and distribution in Alaska, their northernmost range on this continent. Although the first day exceeding 60°F (day-degree base for grasshoppers) occurred three weeks later than in 1961, fifth instar nymphs were collected in the Matanuska Valley by June 20, the first adults on July 20. Adults were found as late as October 2. Oviposition apparently is confined to Hordeum jubatum while Artemisia alaskana seems to furnish only shelter. Hylemya florialis activity, delayed by a wet cold spring, continued throughout September. B. thurngenensis, elsewhere an effective control parasite, gave no control in Alaskan turnip infestations. Asphalt emulsion mulch speeded seed germination but did not prolong the effectiveness of soil treatments such as

heptachlor, chlordane, or endrin. Preplanting soil treatment with diazinon emulsion gave better maggot control in turnips than four other commercially available materials.

A growing list of insect invasions in Alaska call for a wide variety of adapted control techniques. Most active and spectacular during the past year were red turnip beetles (widespread damage to gardens in remote villages), cecidomyiid fly larvae (in mushroom spawn), spring tails (vegetable seeding in Tanana Valley), wireworms (in potatoes), root weevils (in strawberries), English grain aphid (barley and oats), little red louse and short nosed louse (cattle), feather mites and shaft louse (poultry), banded brown roach, clover mite and carpet beetles (households), Tribolium species (in prepared, imported food mixes) and many kinds of aphids (on birch trees and ornamentals). Meadow spittle bug survived in strawberry plantings, an extension of its northern record.

The necessity for insect pollination of named crab apple varieties (except Malus baccata) was again confirmed. Bee swarming in late July was effectively reduced by caging queens, thus diverting the hive to honey production rather than rearing young.

B. Puerto Rico (Mayaguez)

1. Insecticidal Crops. Before the annual African legume, Tephrosia vogelii, can gain acceptance as a commercial source of rotenone, its leaves and stems must contain a certain minimum per cent of this insecticide. A breeding and selection program, now in the F₅ generation, has almost doubled the original rotenoid content and has resulted in production of some selections which average nearly 5 per cent total rotenoids on a dry weight basis. Recent experiments indicate that rotenoid content can be still further increased by preharvest treatments. These include wilting before drying and application of growth-regulating chemicals as sprays. These treatments were found to induce premature yellowing of leaves and to produce a statistically significant increase in total rotenoid yields. Exposure of plants to temperature of 50°F for up to 48 hours did not promote leaf yellowing or increase rotenoid per cent.

2. Drug Crops. The comparison of two species of cortisone yams, Dioscorea composita and D. floribunda, in the fourth and final year of a replicated field trial showed that, although the per cent diosgenin was slightly lower in the former species, its larger tuber size contributed to higher overall diosgenin yields. An increase in average diosgenin content in D. composita of 20 gms. per tuber during the fourth year suggests that harvest times longer than 3 years may be advantageous.

An adequate source of planting material is vital to the success of Dioscorea as a commercial crop. This problem is being attacked in

three ways: (1) propagation from sexual seeds, (2) propagation from 1-node stem cuttings, (3) propagation from tuber pieces. Studies on seed germination show that seeds of diosgenin-bearing species can be stored for at least 2 years and retain a viability of 80 per cent or higher if maintained at a relative humidity between 0 and 30 per cent. Sixty-nine per cent of 1-node stem cuttings from juvenile vines were found to produce roots, as compared to only 2 per cent for similar cuttings taken from mature vines. Tuber pieces whose dormancy was broken by exposure to ethylene chlorohydrin (60-120cc per liter of air space) for 8 hours gave 64 per cent sprouting after 3 months in the field, as compared to 27 per cent sprouting for untreated tuber species. Significantly better sprouting was obtained from tuber pieces cut from plants harvested in July than from those harvested in November.

A biochemical synthesis of additional diosgenin occurs in homogenates of fresh Dioscorea tubers. Optimum yields were obtained with an alkaline pH close to 8.0 and an incubation temperature of around 30°C. The reaction appears to be essentially complete within the first hour of incubation under these conditions. Separation of the steroid components of Dioscorea homogenates by column chromatography was improved by use of a new linear gradient elution technique.

Hybrids of all combinations of D. composita, D. floribunda, and D. spiculiflora, were produced by hand pollination. In comparison with the parent species, these showed poor seed germinability, satisfactory seedling viability, and a modified juvenility stage. These hybrids, along with a putative natural hybrid of D. floribunda and D. composita collected in Oaxaca, Mexico, are being evaluated in the breeding program. Counts of chromosome numbers were made for six new-world species of Dioscorea and for several old-world and African species. These revealed multiples of 9 for the new-world forms (counts of 36, 54, and 72 were found) and multiples of 10 for the old-world types (counts of 40, 60, and 80 being reported). In the African forms, chromosome numbers which are multiple of both 9 and 10 were found.

One hundred and twenty-four new collections of tropical legume seeds were sent to Boston University for assay in an effort to locate additional plant sources of blood agglutinins. The seed-collecting phase of the study has now been completed, with approximately 1500 tests having been made on some 450 different plant species.

3. Food Crops. The size and growth habits of sugarcane make it difficult to spray individual stools for research purposes. To overcome this difficulty a self-supporting, easily movable device, operated from the ground, was constructed. This consists essentially of a truncated, quadrangular metal frame with cloth or plastic covering, topped with a compressed-air spray gun and automatic measuring pipette. With this equipment, up to 20 stools of cane can be sprayed per hour.

Phosfon, a growth regulator, and maleic hydrazide, a sprout inhibitor, were evaluated in field trials on PR 980 sugarcane in cooperation with the Agricultural Experiment Station of the University of Puerto Rico. The applications were made at the end of May, at a time when the seasonal decline in sugar yield had just begun. Neither chemical prevented large seasonal declines in sucrose yields, but Phosfon significantly reduced the drop in sucrose concentration in the juice and maleic hydrazide did likewise, both for sucrose in the juice and sucrose in the cane. Neither chemical caused a reduction in the weight of cane per stool.

4. Plant Introduction and Testing. Two hundred and nineteen new plant introductions were made during the year, including 54 new clones from Ecuador, Haiti, and Jamaica for the Cacao germplasm collection in Mayaguez. Two hundred and forty-six clump offsets of bamboo, 147 packets of seed, 449 plants, and 100 square feet of Zoysia sod were distributed in response to foreign and local requests.

An artificial medium was developed which gave a high percent germination of tomato pollen, but pollen tubes did not grow sufficiently long for comparison with normal pollen tubes. It is hoped this technique can be perfected for in vitro studies of self- and unilateral-incompatibility. The wild, green-fruited tomato, Lycopersicon hirsutum, is admirably suited for genetic studies of compatibility, but is extremely sensitive to changes in day length. The plants develop short internodes and flower abundantly during the 11-hour days of winter in Puerto Rico, but develop long internodes and do not flower during the 13-hour days of summer. It has been found that one of these factors, plant height, can be controlled by application of Cyclocel to potting soil. This is being used in combination with short-day treatments in an attempt to induce normal growth and flowering of this species during the summer months.

5. Spice and Special Crops. The culture of Vanilla in Puerto Rico has been limited by the occurrence of a serious root-rot disease. Efforts to control the disease by cultural treatments and by breeding have been hampered by a lack of genetic diversity in the few clones available for study. This past winter, in cooperation with the New Crops Research Branch, an expedition was organized and sent to the principal Vanilla-producing areas of Mexico. A total of 2950 clones of three species was secured and successfully transported to Puerto Rico. Since Mexico is thought to be the botanical center of origin of Vanilla, these new introductions should provide a wealth of genetic material for future investigations.

Samples of soil and diseased Vanilla roots from an area known to have a high disease incidence were collected and examined for nematodes. No parasitic forms were found in the roots or soil

examined, suggesting that nematodes are not an important agent in the injury-disease complex causing the root-rot disease of Vanilla.

All native Piper species (of which there are some 8 or 10) appear to be highly resistant or immune to the collar-rot disease of commercial black pepper (Piper nigrum). In an attempt to avoid this serious disease problem, imported black pepper clones have been topgrafted to as many of the native Piper rootstocks as possible. These graft unions have been successful to varying degrees. Some have grown and remained alive for over 2 1/2 years and have flowered and set some pepper fruits. Even in the most compatible combinations, however, growth of the scions has been slow and leaves have tended to be small and wrinkled. Perhaps the most curious and striking effect of incompatibility has been the failure of the scions to form holdfast roots which anchor the vine to its supports. Soil samples collected around the roots of healthy and diseased black pepper plants from four locations were screened for nematodes at the Agricultural Experiment Station of the University of Puerto Rico. The reniform nematode (Rotylenchulus reniformis) was the most abundant species observed. This nematode, known to be a plant parasite, was found in samples collected around diseased plants in all cases. The constant association of this species with diseased plants suggests that it may be a contributing factor in the development of the collar-rot disease of black pepper. Lesion and spiral nematodes (Pratylenchus and Rotylenchus spp.--two ordinarily aggressive plant parasites) were found in some of the samples, in association with both diseased and healthy plants. Dagger and needle nematodes (Xiphinema and Longidorus spp.) were found in a previous screening of soil samples collected in the vicinity of diseased plants.

6. Plant Diseases. Application of two herbicides, Karmex DW and 4 (MCPB), to six varieties of wheat and five varieties of oats 12 and 25 days after planting had no effect on the resistance of the cereals to wheat stem-rust Race 15B-59D or oat crown-rust Race 264. With the exception of a local grass (Echinochloa colonum), effective weed control was noted with 4, 6, and 8 oz./A. of Karmex and 1 or 2 lb./A. of 4 (MCPB) 2 1/2 months after application.

7. Winter Testing and Seed Increase. This past winter an initial supply of only 18 pounds of the new cyst nematode resistant Black Lee soybean, supplied by the Oilseed and Industrial Crops Research Branch, was increased to 974 pounds in winter plantings at Mayaguez. In order to prevent premature flowering during the short days of January and December, it was necessary to provide night lighting to the 1/2 acre seed-increase plot. Light intensities of 2- to 5-foot candles were used for 4 hours each night for a period of 6 weeks, beginning immediately after seedling emergence. When the plants had reached a height of approximately 2 feet, the night lighting was discontinued and flower induction occurred. Seed

was shipped to the mainland during early April for spring plantings in nematode infested areas of the southern and eastern states.

The mild climate of Puerto Rico permits the growth of winter generations there, thus expediting research programs in the United States. This past season, 82 tobacco breeding lines supplied by the Tobacco and Sugar Crops Research Branch were evaluated for disease resistance, growth habit, leaf size and shape, and alkaloid content. Selfed seed of over 450 selected plants was collected and sent to the States in time for spring plantings there. In addition, 90 different treatments of growth retardant chemicals for sucker control were applied to topped plants of two commercial tobacco varieties, Burley 37 and SC 58. These treatments were compared with maleic hydrazide at Mayaguez to obtain precise information on formulations, application rates, toxicity to leaf tissue, and effectiveness. Final trials based on the information obtained during the winter are being carried out at the various commercial tobacco growing areas of the United States this spring and summer.

The 1962-63 winter testing of wheat and oats for resistance to rust consisted of 9080 oat and 6576 wheat entries. There was a wheat stem-rust nursery for each of the following races: 15B-62D, 56, and biotypes of 15B located, respectively, at Isabela, Mayaguez, and Ponce (Fortuna). Two of the four oat nurseries were for crown-rust races 264 and 290 located at Isabela and Ponce. The other two nurseries were for the combination of oat stem-rust races 6A and 13A at Mayaguez, and biotypes 6AF and 6F of race 6 located at Lajas.

As in past years, this station has cooperated in increasing seed of Crotalaria juncea (sunhemp) for special fiber trials conducted by the New Crops Research Branch in the continental United States. Approximately 50 pounds of seed was collected and sent to Beltsville in February.

C. Virgin Islands

1. Soil Science. A plot of sugarcane on Fredensborg clay soil was plowed, harrowed and raked when so wet that it suffered considerable compaction. One third of the plot was seeded to okra immediately, one third was rototilled once before planting, and the last third was rototilled twice. Replicated, randomized blocks of each treatment were used. Okra on the compacted soil very significantly outyielded the rototilled plots, and the once tilled plots outyielded those tilled twice. It appears that this particular soil can be worked when wetter than optimum.

2. Animal Science. Calculations from farmers' records show that the net cost of producing milk under the common, local system (once a day milking by hand, with little or no concentrate fed) averages

15 cents per quart. Farmers milking twice a day by machine and feeding adequate concentrates produce milk at an average cost of 11 cents per quart. Returns per acre of pasture from the old system are approximately \$20 per year, and from the up-to-date system \$100. Since doubling pasture production by fertilization costs \$40 per year, the old system operator cannot afford to fertilize and the modern dairyman cannot afford not to.

It has been demonstrated that the tropical cattle tick (Boophilus microplus) which transmits anaplasmosis and piroplasmosis of cattle, can complete its life cycle on the white-tailed deer. However, the degree of infestation on the captive deer held in small pens has decreased so much during the period of the study that the effect of a small wild deer population in an area where a tick eradication program is underway is still not clear. It appears possible that under natural conditions the ticks might not be able to maintain themselves permanently on deer if the possibility of reinfestation from cattle were eliminated.

3. Plant Science. The cost of growing cucumbers in the Virgin Islands is greater than the returns, whatever time of year they are produced, and whether they are grown for slicing or pickling. Development of new varieties resistant to local strains of downy and powdery mildew, and discovery of an effective insecticide, use of which could safely be continued during the harvest, might make a profit possible, though it would probably not be as high as for sugarcane, papayas, or dairy pasture.

A "Growers' Association" has been formed in St. Croix, with about 40 paid-up members. Three avocado orchards have been set out, and about 6000 'solo' papaya trees have been planted on 12 acres by members within the past four months, and land is already being prepared and trees produced to about double this quantity. There is a considerable demand for quality papayas, not only locally and in the United States, but also in Puerto Rico where the presence of mosaic and bunchy-top diseases makes production of papayas difficult.

4. Home Economics. Ninety-five demonstrations of cooking, sewing, home decorating, etc., were made to clubs and other groups of adult women. Demonstrations and project supervision were also given to the girls in four 4-H Clubs in St. Croix and those in St. John.

5. Extension Service Activities. In addition to extension activities included above, 37 news releases and newsletters were issued, 85 radio programs prepared and presented, 293 farms and homes visited, 57 demonstrations and 4 short courses conducted, and 9 farmers meetings held.

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CORDAGE FIBERS CULTURE, BREEDING AND GENETICS,
DISEASES, AND VARIETY EVALUATION
Crops Research Division, ARS

Problem. The United States depends upon foreign producers for its jute, kenaf, abaca, sisal, and henequen. The development of a domestic production would be well received by United States manufacturers. Kenaf has proved to be an acceptable substitute for jute and can be grown in many of the southern states. Sansevieria which can be substituted for abaca and sisal can be grown in southern Florida. Some jute manufacturers are apprehensive about a continued source of supply because of the unrest in India, Pakistan, and China. The price of the imported fiber determines the feasibility of producing the crop in the United States during peace time. Before a production industry can be established, additional information is needed on cultural requirements as they affect yield and quality of fiber. There is need for the development of kenaf varieties that are resistant to root-knot nematode, a most serious pest in all areas where the crop is grown. Breeding behavior of the fiber type species of hibiscus needs to be studied in order to transfer desirable genes from one into another. Retting methods need to be further studied in an attempt to reduce the cost of processing and to improve fiber quality. The sansevieria breeding program needs to be continued to improve the present outstanding hybrid Florida H-13. Emphasis is being placed on breeding for higher fiber yields and for more cold tolerance.

PROGRAM

The Department's program with cordage fibers involves diseases, varietal evaluation, genetics, breeding, and culture.

The work is cooperative with the University of Florida Everglades Experiment Station and the Agricultural Engineering Research Division at Belle Glade, Florida.

The federal scientific effort devoted to all phases of research is 5.5 professional man years. Of this number 1.0 is devoted to culture; 2.5 to breeding and genetics; 1.0 to diseases; and 1.0 to varietal evaluation.

PROGRESS

A. Breeding and Genetics

1. Kenaf. The most important factor limiting the growth of kenaf (Hibiscus cannabinus) is root-knot nematodes. Early attempts were made to transfer resistance to H. acetosella into kenaf resulting in sterile hybrids. Subsequent experiments indicated that somewhat fertile hybrids could be produced by crossing H. acetosella with H. radiatus and by crossing H. cannabinus with H. radiatus. This suggested the possibility of transferring the nematode resistance of H. acetosella into H. cannabinus by using H. radiatus as a bridging species. In 1963, results show that the resistance of H. acetosella was dominant over the susceptibility of H. radiatus and H. cannabinus and thus it appears possible to transfer the resistance of H. acetosella into H. radiatus.

Additional new hybrid combinations were obtained by crossing H. cannabinus-acetosella 6x with H. radiatus and by crossing H. cannabinus-acetosella 6x with H. diversifolius. The hexaploid radiatus progenies were backcrossed to H. radiatus and to H. cannabinus. There is a good possibility that the nematode resistance of H. acetosella can be transferred into H. cannabinus.

A successful cross was made for the first time between H. sabdariffa and H. meeusei. A vigorous plant was obtained which was almost completely sterile. However, enough pollen was obtained from the plant to backcross with H. sabdariffa and to out-cross to H. acetosella. Some seed were obtained from each cross.

2. Sansevieria. Data were collected on 6 diagnostic characters in the species S. trifasciata, S. deserti, their F₁ and F₂ hybrids, and the backcross progeny. Inheritance patterns can be interpreted in simple Mendelian terms. Even though the two species differ very distinctly morphologically and physiologically, recombination has not been suppressed.

B. Diseases

1. Kenaf. Emphasis was placed on root diseases affecting kenaf. Pythium irregulare and P. splendens were found to be pathogenic to kenaf. The former is active on older roots while the latter is particularly virulent to germinating seed.

An NPK soil fumigant factorial was designed to study the effect on P. splendens on kenaf. The presence of nitrogen encouraged the growth of the fungus while the presence of phosphate was highly

significant in retarding the growth of the fungus. The fumigant, Chloropicrin, gave the best control of P. splendens and was especially effective on plots receiving phosphate.

In earlier studies, Botrytis sp. attacked kenaf and roselle (H. sabdariffa) after a period of low temperatures. It had not been considered a serious disease; however, it became very destructive in 1962-63. Results indicate that low temperatures apparently encourage the development of the sexual stage of the fungus and produces the primary inoculum. The fungus grows, sporulates and invades tissue more rapidly at 66 to 75° F. than at 40 to 60° F; nevertheless outbreaks occur after cold weather. Botran was more effective in controlling the disease than were thiran, Dyrene, or ferbam.

C. Culture

1. Sansevieria. Florida H-13, the superior yielding, cold tolerant hybrid, developed as a result of a cross between S. trifasciata and S. deserti was released by the Crops Research Division and was described in the Florida Agricultural Experiment Station Circular S-141.

A few F₂ clones of S. trifasciata x S. deserti gave a higher percentage of fiber than either parent. They are being increased and will be put in a yield trial in 1963.

In past studies, the application of lime increased yields of sansevieria. A fertility experiment which included N, K, lime and gypsum was designed to determine if increased yields were due to an increase of pH or to the presence of calcium. Yields were significantly increased with the application of either 500 pounds of slaked lime or 500 pounds of gypsum per acre, but were depressed when a combined application of lime and gypsum was used.

The experiment to determine the effect of age on yield of the sansevieria hybrid, Florida H-13, continued. The yield of fiber from plots 6-1/2 years old was greater than plots 5-1/2 years old. However, annual average yields declined to 753 pounds per acre at 6-1/2 years as compared with 1175 pounds per acre up to 3-1/2 years.

The age of leaves at the time of planting had no effect on fiber yield.

A sansevieria spacing experiment was designed in which plants were spaced 4 inches and 8 inches and rows were spaced 8 inches and 16 inches. The 4 inch plant spacing with the 8 inch row spacing produced a significantly greater number of plants. The effect on fiber yield will be determined later.

2. Kenaf. Duplicate yield trials of 16 varieties of kenaf were planted on land known to be infested with root-knot nematodes and on land that had been in a solid stand of ramie for 8 years. Ramie is not affected by root-knot nematodes. Results showed significant differences between varieties in yield of fiber. However, there was no significant difference in yield between the two locations indicating that root-knot nematodes were present in great enough numbers in the ramie planting to reduce yields of kenaf.

The effect of source of nitrogen on kenaf was studied. Nitrogen was applied at the rate of 150 pounds per acre in three applications. The following sources were used: ammonium nitrate, sodium nitrate, nitrate of soda/potash, calcium nitrate, uramon, millorganite, calcium cyanamide, guano, and leather shavings. The best source, calcium nitrate, produced 2,300 pounds of fiber per acre while ammonium nitrate, uramon and millorganite produced 2,000 pounds, 1,900 pounds, and 1,500 pounds, respectively, per acre.

3. Sisal. The application of 1,000 pounds of slaked lime and 300 pounds of K_2O per acre produced a significantly greater number of leaves in sisal than 500 pounds of lime and 200 pounds of K_2O .

4. Ramie. Planting stock of superior clones was maintained. Fiber samples from degumming studies were evaluated in the laboratory.

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MINT, HOP, GUAR, AND CANAIGRE CULTURE, BREEDING, DISEASES,
AND VARIETY EVALUATION

Crops Research Division, ARS

Problem. Verticillium wilt of mint, estimated to cause an annual loss of up to 20 percent in Indiana, Michigan, Oregon, and Washington, is the most serious problem in mint production. A single variety within the species Mentha piperita produces peppermint oil acceptable to the trade. Attempts to breed acceptable varieties resistant to the disease have been unsuccessful to date. Control of the disease through cultural treatment or the use of fungicides seems the only practical solution in some areas of production. Objectives of hop research include breeding improved varieties with higher yield, improved brewing qualities; greater resistance to downy mildew, Verticillium wilt and virus diseases; and the development of practical methods of disease control. Guar, a summer legume, grown as a soil-improving crop in lighter soils of the Southwest, is a source of industrial gum and a high protein livestock feed. Available germ-plasm needs to be screened for agronomic, disease resistance, and yield characteristics. There is no record of artificial hybridization in guar although plant progeny populations have been observed segregating in a simple genetic ratio indicating that natural crossing must occur. A suitable method for controlled hybridization is needed to improve the possibility of developing superior varieties. Practical disease control is an important problem in guar improvement. Research on canaigre was completed with the harvest of last season. A few breeding lines remain to be planted in permanent germplasm banks.

PROGRAM

The Department has a continuing long-term program involving geneticists, plant pathologists, agronomists, and chemists engaged in both basic studies and the application of known principles to the solution of growers' problems. Hop research is centered at Corvallis, Oregon in cooperation with the Oregon Agricultural Experiment Station, and at Prosser, Washington in cooperation with the Washington Agricultural Experiment Station. Mint research is conducted at Corvallis, Oregon, and Lafayette, Indiana in cooperation with the Oregon and Indiana State Agricultural Experiment Stations, and at Beltsville, Maryland. Guar breeding and testing is conducted at College Station, and Iowa Park, Texas, and Stillwater, Oklahoma in cooperation with the Texas and Oklahoma Agricultural Experiment Stations.

The Federal scientific effort devoted to research in this area totals 5.4 professional man-years. Of this number 2.2 is devoted to breeding and genetics; 0.9 to diseases; 0.5 to variety evaluation; 1.4 to culture and physiology; and 0.4 to program leadership.

PROGRESS

A. Breeding and Genetics

1. Mint. Oil quality of wilt-resistant selections from the cross Mentha piperita X M. crispa was determined by a commercial cooperator and found to be unsatisfactory as a substitute for oil from M. piperita. Further tests will be made to determine if the oil has a market potential. A number of varieties of M. arvensis v. piperascens, and hybrids with M. crispa were grown at Sunnyside, Washington for preliminary evaluation. Final quality tests are not yet available.

2. Hops. Breeding for resistance to downy mildew progressed during the year. About 7000 seedlings were grown from crosses of wild X domestic, wild X wild, and domestic X domestic hops. These seedlings were given a severe crown inoculation test in the greenhouse and 287 plants rated as highly resistant were selected and planted in the nursery for further study and evaluation. A nursery planting of 277 individual plants resistant to downy mildew were planted under trellis for preliminary evaluation. Eleven selections with a high level of resistance were transferred to an observation block. Although Verticillium wilt has not become a serious hop disease in the United States, it is known to cause large losses in Europe. Three wilt-tolerant varieties were introduced from the East Malling Research Station in England and are being observed for disease resistance, yield, and quality. A preliminary planting was made of 11 selections, two commercial varieties and 128-I that have received favorable comment from industry brewing personnel, have marked resistance to downy mildew, or excellent vigor. Final data were recorded from a study of inheritance of several characters in hops. Variables studied include alpha-, beta-, and total hop-acids, flowering dates, and downy mildew resistance.

3. Guar. The guar breeding program entered a new phase in 1962 with emphasis on selection and evaluation of pure lines from heterogeneous populations rather than upon evaluation of introductions. Groehler-1-3, a selection from the Groehler variety, is resistant to both Alter-naria and bacterial leaf-spot diseases, and produced the highest yield where these diseases were serious. At one location where diseases were not a factor, its yield was not significantly different from the highest yielding entries. Two other selections from Groehler were outstanding in yield. A number of selections with a distinct fruiting type have been selected. These have numerous branches with small fruiting racemes along the main stem and branches. This type may suffer less shattering loss in harvesting. Thirty-one pubescent plants proved to be natural hybrids, their progenies segregating in a ratio approximating 3 pubescent to 1 glabrous. A study of natural crossing in the field ranged from 0.0 to 7.7 percent outcrossing. Thus it seems evident a practical method of effecting artificial

hybridization can be achieved.

4. Canaigre. A nursery of 185 entries recently collected in southern Arizona or Sonora, Mexico were grown for observation. Percentage tannin and purity were determined and 29 entries were added to the germplasm bank. Triplicate plantings of 183 vegetative lines were made at remote points in the wild within the Tonto National Forest.

B. Diseases

1. Mint. In crop rotation studies in which mint followed 3 years of corn or sudan grass both the incidence and severity of Verticillium wilt was significantly reduced. It is important that corn rather than sudan grass may be used effectively in a crop rotation since it allows the land to be used for production of a more profitable crop. Soil fumigation was effective in reducing losses from wilt in both Indiana and Oregon, but heavy rates were required and cost of chemical makes its use impractical at present. Greenhouse tests of systemic growth regulator chemicals applied as a foliar spray showed a wide range of host response. Results ranged from no infection to 100 percent infection compared with 90 percent infection for the untreated check. Several of the chemicals were non-phytotoxic. Field testing will be required to evaluate the practical potential of these chemicals.

2. Hops. Three commercial hop varieties, Fuggle, Late Cluster, and Brewers Gold, and seven lines from the advanced field tests showed high tolerance to four prevalent strains of Verticillium wilt in greenhouse tests. A strain of Verticillium obtained from Fuggle hops in Oregon caused the most infection. A strain from mint was less pathogenic than the hop strain. The widely prevalent potato strain did not infect hops systemically.

Data on downy mildew reaction were obtained on 128 female and 59 male lines in the breeding block. On the basis of incidence of systemic shoot infection 82 were resistant, 46 susceptible and 59 intermediate. The collection of wild American hops was tested for downy mildew resistance. None was immune to systemic infection, and most clones were highly susceptible. One clone showed a high degree of resistance.

3. Guar. Diseases were a major factor in determining seed and forage yields in guar variety trials in Texas and Oklahoma. Alternaria leaf spot and a bacterial blight, probably caused by Xanthomonas cyamophagus, caused complete loss of susceptible guar lines at College Station, Texas, and were severe at Iowa Park, Texas, and Perkins, Oklahoma. A new selection, Groehler-1-3, was the most resistant line or variety tested. One introduced line, P.I. 179930, was resistant for the second consecutive year.

C. Varietal evaluation

1. Hops. Ten varieties from Oregon, eight from Idaho, and four from Washington were evaluated in the chemical laboratory and later hand evaluated by members of the U. S. Brewers Association Fixed Hop Committee. The experimental variety 128-I rated outstanding in Oregon, but only average in Idaho. Two other Oregon varieties rated good as did one variety from Idaho and one from Washington. Only two varieties rated below average.

A study was initiated to provide an accurate description of morphological, chemical, plant, and brewing characteristics of hop varieties now grown in the United States to provide a means of varietal identification useful to hop growers, brewers, and agronomists; to have descriptions of dried hop cones that will indicate the origin and potential brewing value of a particular lot of hops; to give a detailed description of hop varieties that will be of value to hop breeders.

2. Guar. Seed yield data were obtained from seven locations in Texas and Oklahoma. Groehler-1-3 produced the highest average yield of 645 pounds per acre in the four tests where it was grown. Where disease was a factor in determining yields, Groehler-1-3 was outstanding and ranked with the best. Another selection Groehler-1-2 was earlier in maturity than Groehler-1-3, and may eventually prove superior. A modest seed increase is planned by both the Texas and Oklahoma Stations in 1963 for possible release as a new variety.

D. Culture and Physiology

1. Hops. Foliar applications of 2, 5, and 15 ppm. of three formulations of gibberellic acid (GA_3) applied at the 5-foot stage did not produce a yield change. This is in direct contrast to the average for the 3-year period 1959-61, when 5 ppm. resulted in an average increase of 300 pounds per acre. In 1962 no differences in alpha- or beta-acid or oil content were noted as a result of application of GA_3 but cone size decreased with increased applications. Further study is required to clarify conflicting results.

Three years' results indicate wide differences in dates-of-pruning and training had no effect on yield, cone size, oil content, or beta-acid of Late Cluster hops. However, data obtained in 1962 showed an increase in alpha-acid content from very early to very late pruning.

First year data on trellis heights of 16, 18, or 20 feet, indicated no significant differences for yield.

2. Canaigre. Final data from an irrigation-fertilizer trial with canaigre were obtained during the reporting year. As a result of 2-years' observation the following conclusions were drawn: Maximum

yield of roots may be expected for any given set of conditions only when soil moisture is constantly adequate to prevent yellowing of lower leaves until normal top decline begins. Adequate irrigation differs with moisture holding capacity of the soil, and may require as few as 4 irrigations with a total of 2 acre feet on a heavy soil to 12 or more irrigations with a total of 4 or 5 acre feet of water on highly porous soils. Water requirements were highest in March, April, and early May in southern Arizona. Tannin content and extract purity of the roots were not affected by irrigation treatment.

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INSECTS AFFECTING MAN, HOUSEHOLDS, AND INDUSTRIAL ESTABLISHMENTS
Entomology Research Division, ARS

Problem. Insects, ticks, and mites are responsible for widespread human misery and certain insects cause heavy losses of food and materials in households and industrial establishments. Many of the same or closely related insects which affect man are also important pests of livestock, thus research on insects in relation to man and to livestock is mutually advantageous. Certain arthropods are vectors of major diseases which annually cause the deaths of millions of humans. Mosquitoes, for example, transmit malaria, dengue, encephalitis, yellow fever, and filariasis. Some of these diseases, as well as other arthropod-borne diseases, occur and are potentially serious problems in the United States but most of them are of more concern in other parts of the world, where troops and civilian personnel are maintained. The military agencies have for many years depended on the research competence in agriculture for answers to their military insect and insect-borne disease problems. Attacks by insects, ticks, and mites frequently prevent farm and forest work, reduce or destroy the value of recreation areas, and even make certain areas uninhabitable. Property values are often depressed and development prevented by hordes of annoying pests. Mosquitoes, bed bugs, and fleas are frequently serious annoyances in homes. Other household insects are of economic importance in homes and industrial establishments because they damage foods, fabrics, and other materials, causing losses of millions of dollars annually. There is a great need for safe, economical insecticides and satisfactory methods for their application that could be used quickly and effectively to control local infestations or outbreaks of pests that annoy man in the field or at home, especially where there are threats of disease epidemics. Improved means for controlling mosquitoes, sand flies, gnats, the imported fire ant, and similar pests, should receive particular attention. More efficient repellents are needed to protect humans, particularly when other means of control cannot be employed. Special efforts should be made to develop systemic materials which, when taken orally, would repel or prevent insects from biting. Sanitation, habitat management, and other non-insecticidal methods of control should be reappraised, and biological control, especially with insect pathogens, needs to be fully explored. New approaches to control, including chemosterilants, antimetabolites, attractants, and radiation require intensive investigation. Studies should be undertaken on the biology, ecology, physiology, and genetics of many important pests affecting man and the household in order to find weak points in their life cycles which might be utilized to improve control efficiency.

The Department has a continuing program involving applied and basic research on insects affecting man, households, and industrial establishments, including mosquitoes, house flies, human lice, bed bugs, gnats, fleas, ticks, mites and other pests of man, and on cockroaches, ants, and several other pests of importance in households and industrial establishments. Research is directed toward the development of more effective insecticides and repellents and involves primary screening of chemicals and field evaluation of promising

materials. Investigations are conducted on the nature of insect resistance to insecticides, on the mode of action of insecticides, on the effects of radiation and chemosterilants, on attractants, on factors affecting attraction of biting insects to humans, and on the factors affecting the effectiveness of repellents. Attention is also given to the development of sanitation and management procedures and to biological control methods for mosquitoes, house flies, cockroaches, and several other pests. Studies are conducted in cooperation with the Agricultural Engineering Research and Animal Husbandry Research Divisions to develop physical and mechanical methods of insect control and to evaluate various kinds of traps and devices for estimating and controlling natural populations of flies, mosquitoes, and other pests. Studies are also conducted in cooperation with the Soil and Water Conservation Research Division of ARS and the Bureau of Vector Control, California State Department of Health, on soil and water management procedures to prevent mosquito production in western irrigated areas and in log ponds. The major portion of the program is now conducted at Gainesville, Fla., following a transfer in June 1963 of the investigations from Orlando, Fla., where the research herein reported was carried out. The remainder of the work is done at Corvallis, Oreg., Fresno, Calif., and Beltsville, Md. Close cooperation and evaluation of research data and needs are maintained with the Defense Department through the Armed Forces Pest Control Board concerning studies on insects important to military personnel. Research funds supporting 1 1/2 professional man-years have been transferred from the Defense Department to support research on chemosterilization of house flies. Cooperation is maintained with the World Health Organization on studies developing new insecticides and other methods of control on insects affecting man. The World Health Organization provides financial support (1/2 professional man-year) for studies at Gainesville, Fla., on the development of residual insecticides for mosquito control.

The Federal scientific effort devoted to research in this area totals 20.1 professional man-years. Of this number 3.1 is devoted to basic biology, physiology, and nutrition; 9.1 to insecticidal and sanitation control; 0.7 to biological control; 5.7 to insect sterility, attractants and other new approaches to control; 0.5 to the evaluation of equipment for insect detection and control; and 1.0 to program leadership.

Additional research (2 professional man-years) has been initiated under a grant of P.L. 480 funds (S9-ENT-3) to the Facultad de Agronomia, Universidad de la Republica, Uruguay, on "Investigations on the biology and biological control of the fire ant, Solenopsis saevissima richteri, in Uruguay."

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Basic Biology, Physiology, and Nutrition

1. Mosquitoes. At Orlando, Fla., studies on the biology and behavior of mosquitoes were continued to obtain basic data applicable to the development of new approaches to control, particularly the sterility method. A two-year

study on Anopheles quadrimaculatus biology under field conditions has been concluded. Data have been obtained on population levels, climatic conditions, mating activity, dispersion, and physiological age and insemination rates of laboratory as well as wild strains. Techniques utilizing sterilization by radiation or chemicals along with tagging with radioisotopes have been developed and with these it has been possible to show that colonized A. quadrimaculatus mosquitoes have behavioral, mating, dispersion, and biting traits which differ considerably from the same traits in wild mosquitoes of the same species. The ability to sterilize mosquitoes with chemicals or radiation has offered an excellent tool for basic studies of mosquito biology and mating behavior under either laboratory or field conditions.

Laboratory studies with the salt-marsh mosquito have defined conditions essential for a high degree of mating activity and egg viability, which will permit experiments on chemosterilization of this mosquito. A series of laboratory tests with adult A. quadrimaculatus did not show the presence of a chemical attraction of either sex for the other.

Surveys and studies on the occurrence and biology of Anopheles albimanus were conducted on San Salvador, one of the Atlantic Missile Range Islands. Preliminary data have been accumulated on population levels, breeding sites, insemination rates, and physiological age of wild females.

At Corvallis, Oreg., and Fresno, Calif., studies were continued on the biology and ecology of important mosquito species. In laboratory tests the majority of Culex tarsalis females preferred to feed at the higher of two feeding locations and preferred mice rather than chickens for their source of blood.

A study was conducted on the daily activity of several species of mosquitoes--Anopheles freeborni, Culex peus, Culiseta inornata, Culex incidens, and Culex tarsalis. All species behaved similarly. Both males and females began to move out of daytime resting shelters a few minutes before or after sunset. Only a few males and females (0.3 to 2.8%) remained in the shelters during the night. Adults did not begin to return to the shelters until sunrise or a few minutes later, and all did not return until sometime after 8:00 a.m. Exodus from and return to shelters appeared to be regulated by light intensity, but some other factors such as preconditioning to light cycles, temperature, and humidity may have had some effect since artificial lights did not prevent mosquitoes from leaving shelters eventually, but did change their time and rate of leaving. Use of special crosses of resistant and susceptible strains of Culex tarsalis showed that some of the females mate more than one time and utilize sperm from more than one mating.

Taxonomic studies in California have defined suitable characters for separating dark-winged forms of Aedes dorsalis from A. melanomon and have shown that these two forms coexist in only one area (Solano County). Surveys at Borrego Springs (San Diego County) were made to find isolated populations of mosquitoes for future studies on control through sterilization.

Surveys were continued on biting arthropods of the Humboldt River Basin in Nevada. During April, May, and June, water was plentiful and high populations of Aedes melanimon, A. dorsalis, A. vexans, Culex tarsalis, and Culiseta inornata were found. Mosquito larvae were infected with a microsporidian, Thelohania sp. and a bacterium, both of which are under study as biological control agents. In late July, no floodwater Aedes breeding was observed, but moderate breeding of Culex tarsalis and Culiseta inornata was observed. Studies of mosquito larval populations in typical breeding areas in dairy drains and culverts in California during April indicated the presence of Culiseta inornata, Culiseta incidens, Culex tarsalis, Culex peus, Culex apicalis, and Anopheles franciscanus, but no breeding of Culex quinquefasciatus was found.

Using C-14 labeled TDE, slight but consistent differences in its rate of degradation have been noted in susceptible and resistant strains of Culex tarsalis indicating that the main source of resistance to this chemical is increased degradation of TDE to FW-152 and water soluble metabolites through an oxidative rather than a dehydrochlorination mechanism. Further tests with DDT and TDE have shown that analogs of DDT which block metabolism via the oxidative pathway overcome resistance to DDT and tend to confirm the hypothesis that resistance to DDT and TDE in tarsalis is a more rapid oxidative metabolism of the insecticide.

2. House Fly. At Orlando, Fla., research on control or eradication through the use of sterilization by radiation or chemicals has shown that considerable gaps exist in knowledge of the biology and mating behavior of house flies. Both males and females must undergo a sexual maturation time of at least 16 hours with males and a minimum of 24 hours with females. Once the mating drive has started, males will attempt to "strike" or mate with both males and females and certain inanimate objects, although they "strike" more readily and frequently with females. This fact along with experiments in a large cage-type chemotactometer suggested the presence of some type of a female sex attractant of a low order. Imperfect sex recognition in the male combined with a low order sex attractant in the female would account for the fact that males attempt to mate with either sex and result in the higher ratio of male-to-female "strikes." Males or females which had their wings removed were able to mate with individuals of the opposite sex with wings. Amputation of more than one pair of legs from the male prevented mating, while amputation of only one pair of legs impeded but did not prevent mating.

Tests were run to determine the actual time house flies remain in copula after initial seizure. Of 61 mating pairs that were trapped and observed, the shortest mating period was 44 minutes and the longest, 96 minutes. The average was 60 minutes. After mating periods of only 1 and 2 minutes, no sperm were found in females, but after 3 to 5 minutes some spermathecae contained a few sperm and some were completely filled. For periods of 10 to 76 minutes spermathecae were filled to capacity with sperm with only one exception.

Observations on the natural sterility of 86 female house flies reared in the laboratory gave an average of 94.4 eggs per female of which 89% hatched. The number of eggs laid varied from 12 to 186 per female. Some flies fed within an hour after emergence, others did not feed until later, and some did not feed until they were 16 to 18 hours old.

At Corvallis, Oreg., studies were continued on the physiology of resistant and susceptible house flies. Isolan-resistant house flies have decreased ali-esterase activity and increased Isolan-detoxifying enzyme. Techniques using high-speed centrifugation have been developed which concentrate enzymes important in metabolizing or conferring resistance to organophosphorus compounds. Experiments with malathion-resistant house flies indicate that the nature of the alkyl groups attached directly to the phosphorus atom is the major factor in resistance. Thus, the primary cause of resistance is probably associated with the rate of recovery of the phosphorylated detoxifying enzyme after poisoning, rather than to an increased ability to cleave the toxic molecule per se.

3. Eye Gnats. Colonization of Hippelates pusio over several years has not induced behavior or mating changes in the colonized individuals. In tests to determine the best combination of CSMA and vermiculite for rearing pusio, a ratio of 1:1 was found to be the most favorable.

An extensive study of the male reproductive system of pusio has been completed. Descriptions of the structure and function of the parts of the male reproductive system--vas differens, accessory glands, ejaculatory duct--have been made. The development of testes and sperm has been followed as males were aged. Tests with males of various age showed that mating did not have any gross observable effect on the testes. However, the density of spermatozoa in the ejaculatory duct increased with age.

Research with female pusio has shown that follicular development within the ovaries of both mated and unmated females follows closely the five stages described for mosquitoes. When the follicles of mated females were in stages IV and V the spermatozoa were always in the bursa. During other follicular stages, the spermatozoa were always in the spermathecal ducts.

Unmated pusio females were capable of laying infertile eggs; however, they required a longer preoviposition period and produced slightly fewer eggs than mated females. Fertile eggs could be stored for 14 days with no indications that storage reduced their viability. High humidities (70% R.H. and over) were more favorable for survival of and egg production in adult gnats.

4. Ants. Information on the distribution, biology, and economic importance of the fire ant, Solenopsis saevissima richteri, in Uruguay has been provided in a report from investigators working in that country under P.L. 480 project (S9-ENT 3). The abundance of ants was greatest in cultivated areas. Damage to mowing equipment, rice field levees, and cyclamen bulbs and stinging of personnel working in areas where the ants occur, were reported. This ant was

associated with 22 species of plants and many insects other than ants were found in the mounds. Possible predation or parasitism of this ant by a mite, a jumping spider, a chalcid wasp, and another species of ant occurred.

B. Insecticidal and Sanitation Control

1. Mosquitoes. Studies were continued at Orlando, Fla., to find new and effective insecticides for the control of mosquitoes. In screening tests with Anopheles quadrimaculatus larvae, 28 of 81 compounds tested were rated Class IV in effectiveness. Four of these compounds--American Cyanamid CL-18133, Stauffer B-10046, Stauffer 8-10094, and Bayer 47940--were highly effective, killing 100% of the larvae at concentrations of 0.05 p.p.m. to 0.01 p.p.m. One hundred and nine plant extracts were also screened for toxicity, but none of these were toxic at low concentrations. Of 83 compounds tested against female Aedes taeniorhynchus in wind tunnel tests, 7 were as effective as the standard, malathion. Dimethrin in granular formulations was effective in laboratory and field tests as a larvicide against Anopheles quadrimaculatus, Aedes aegypti, Aedes taeniorhynchus and Culex quinquefasciatus mosquitoes. A mixture of DDT and anti-resistant compound was no more effective than DDT alone against C. quinquefasciatus larvae.

Field tests were conducted in the Panama Canal Zone to determine the effectiveness of some of the more recently developed insecticides against mosquitoes and to establish a program whereby military entomologists in the field can assist in conducting field evaluations of promising insecticides. Selected insecticides were tested against natural populations of mosquitoes by aerial application, against caged insects by dispersal with the Army cold fogger, and as residual insecticides in horse-baited traps. In tests with caged insects and ground fogging equipment, naled and malathion gave effective control of adult mosquitoes, but DDT did not. In aerial applications, none of these insecticides gave satisfactory control because of dense jungle canopy and migration of mosquitoes into the test plots; however, DDT gave as good control as naled and malathion. Evaluation of the residual deposits has not been completed.

In Florida, extensive screening tests to evaluate new compounds as residual treatments for control of malaria mosquitoes were continued. Among the most effective materials or mixtures tested were Bayer 39007, Bayer 39007 plus Monsanto CP-16226, Bayer 41831, Bayer 37344, Bayer 25141, Hercules 7522H, Hercules 7522H plus piperonyl butoxide, Hercules 7522H plus Monsanto CP-16226, Hercules 7527G, Hercules AC-5227, Hercules AC-5227 plus piperonyl butoxide, Hercules 9699, Monsanto CP-16226, Monsanto CP-40273, Stauffer N-2230, Stauffer 2310, Sevin plus synergist, Upjohn U-12927, and Zectran, all of which were at least 70% effective at 100 mg./sq. ft. through 48 weeks of aging.

At Fresno, Calif., and Corvallis, Oreg., studies on the development of insecticides for mosquito control were continued. In the laboratory 7 of 9

chemicals tested against Culex tarsalis adults in wind tunnel tests were more effective than malathion. In tests with C. tarsalis larvae, eight compounds were highly effective, killing 100% of the larvae at concentrations of 0.05 p.p.m. to 0.0025 p.p.m. In small plot field tests against larvae of snow pool mosquitoes, Bayer 29493 and parathion were the most effective of the compounds tested and Hercules AC-5727, malathion, carbophenothion, and naled gave good control (92% or better). In treatment of log ponds for mosquito control, Bayer 29493 in granular formulation at 0.1 lb./acre gave excellent control and showed some residual effectiveness.

2. House Fly. Research was continued at Orlando, Fla., to develop more effective insecticides and other methods and materials for the control of house flies. Twenty-three new compounds were tested as space sprays in a wind tunnel against the regular susceptible colony and the Cradson (multi-resistant) colony. Fourteen of the compounds were more effective against both the susceptible and resistant colonies than the standard, malathion. New insecticides were also evaluated as residual treatments against female house flies from the regular or Cradson colonies. The criterion of effectiveness was the number of weeks of aging during which the residues remained effective in killing house fly females. Against susceptible house flies Hercules AC-5727 alone and Bayer 39007 alone gave kills of 90% for one week, but in combination with Monsanto CP-16226, their effective periods were extended to 8 and 12 weeks, respectively. The effectiveness of Hercules 7522H was also extended from 1 to 12 weeks with the addition of Monsanto CP-16226. Against flies of the Cradson colony, all of these formulations failed before the fourth week. Against the regular colony Bayer 29952 and Bayer 30237 were effective for 64 and 56 weeks. Stauffer N-2230 and Stauffer N-2404 were 100% effective throughout 48 weeks of aging against the susceptible colony, but they were ineffective against the Cradson colony. General Chemical GC-3583 was still 100% effective after 96 weeks against the regular colony and Monsanto CP-40294 was effective for 24 weeks against the Cradson colony. Against house flies from the susceptible colony, General Chemical GC-4072 was 100% effective for 96 weeks as an acetone solution and Stauffer N-2310, Bayer 39197 and Monsanto CP-40273 for 48+ weeks. As wettable powder, DDT was more than 90% effective for 48 weeks, Bayer 25141 for 48 weeks, Bayer 34098 for 40 weeks, Hooker HRS-1422 for 32 weeks, and Bayer 32651 for 28 weeks.

Residual tests (deposits of 100 mg./sq.ft.) were conducted with emulsions of Diazinon, Baytex, and dimethoate against house flies in Florida dairy barns. The diazinon treatment failed to give satisfactory control as early as the first day after treatment. Baytex gave 97% control for 5 days and from 79% to 88% control through 14 days. Dimethoate gave controls ranging from 80% to 96% for 6 weeks, when the test was discontinued.

Windtunnel tests have been conducted in Florida to compare the susceptibility of house flies from the regular colony and eight experimental colonies to various insecticides. House flies of the experimental colonies showed high levels of resistance to one or more of the following insecticides: DDT,

malathion, parathion, Co-ral, and Sevin. A proposed experiment for the eradication of house flies on one of the Atlantic Missile Range Islands will employ the use of malathion baits with the simultaneous release of malathion-resistant, chemosterilized flies to reduce the fertility of that segment of the population not killed by the bait.

At Corvallis, Oreg., extensive studies were continued on the development of synergists that have overcome resistance to organophosphorus insecticides in both house flies and mosquitoes. Of some new types of compounds screened, results indicate that diisopropyl or dibutyl substituents would be most satisfactory. Selection of house flies with combinations of malathion and synergists is being carried out to determine if resistance to the combinations can be developed.

3. Cockroaches. Research was continued at Orlando, Fla., to develop new insecticides for controlling cockroaches. Of 50 new compounds screened as contact sprays, 16 were promising and gave 100% mortality in 24 hours at a concentration of 0.5%. Of 10 new compounds screened for residual effectiveness against normal German cockroaches, 8 caused 100% mortality in 24 hours after a 30-minute exposure to fresh deposits of 100 mg. per square foot. Two materials--Stauffer N-2230 and Stauffer N-2404--tested as residues were superior to the chlordane standard and caused 100% and 85% mortality, respectively, of German cockroaches after 8 weeks of aging.

Laboratory tests were conducted with Kepone and mirex baits with American and resistant and susceptible German cockroaches. Though effective in killing cockroaches, the two baits were much slower acting than the standard Dipterex bait. However, in a field test over a period of weeks, the Kepone bait gave a high degree of control of American and German cockroaches. Preliminary studies with DDVP as a vapor toxicant against cockroaches indicate that it would require an exposure period of greater than 24 hours to produce 100% kill with a concentration of 1.0 microgram of DDVP per liter of air.

The repellent action of 5 insecticides commonly used as insecticides for cockroach control was evaluated. Chlordane, diazinon, ronnel, and malathion did not function as repellents to the degree that they failed to be effective toxicants. The combination of pyrethrins and piperonyl butoxide repelled cockroaches.

4. Body Lice. Research was continued at Orlando, Fla., to develop more effective insecticides for the control of body lice. Of 299 compounds screened, 84 were rated Class IV and IVA in toxicity and 59 were Class IV and IVA in speed of knockdown. Of materials tested as synergists for Sevin against body lice, none were more effective than the standard sulfoxide, but 2 of them extended the residual effectiveness of Sevin slightly more than sulfoxide did. In patch tests, four compounds--Bayer 39007 and Hercules 9699, Hercules 9326 and California Spray RE-5305-3--were more effective than the standards (DDT and lindane).

Extensive sleeve tests on human subjects were conducted with combinations of Sevin and sulfoxide to develop an effective louse powder. A powder containing 4% of Sevin plus 2% of sulfoxide was 100% effective for 20-21 days after treatment and 95%-98% effective 27-28 days after treatment. A powder containing 2% of Sevin plus 2% of Sulfoxide was 100% effective for 17 days after treatment and 81% to 91% effective 27 days after treatment. The standard malathion powder was 100% effective for 24 days and 97% to 98% effective for 27-28 days after treatment. Cholinesterase levels of the human subjects who wore the treated sleeves remained about the same as before treatment.

Studies were continued on the development of resistant strains of body lice and their susceptibility to various insecticides. Strains that are slightly to highly resistant to one or more of the following compounds--DDT, lindane, Sevin, malathion, and activated pyrethrins--have been developed and studied. No evidence of the development of resistance to one compound with resultant development of susceptibility to another compound has been found.

5. Mites, Ticks, and Fleas. Screening tests were conducted in Florida to find new insecticides effective against fleas. Of 33 compounds tested against oriental rat fleas, 15 were at least as effective as the standard, malathion, in that they caused complete mortality of fleas exposed to residues which had been aged for 12 weeks.

To evaluate compounds against oriental rat fleas under conditions similar to natural situations, a procedure has been developed for the evaluation of the better compounds found in screening tests. The technique involves the application of insecticide dusts to soil and the subsequent exposure of fleas to the treatment. In this type of test Bayer 37344 was the most effective of 9 compounds tested. It caused 100% mortality of fleas exposed to concentrations in dust as low as 0.1%. DDT, Bayer 29493, Hercules AC-5727, lindane, diazinon, and malathion caused 100% mortality at concentrations as low as 1%. At a concentration of 5%, Bayer 39007 and Hercules 7522C produced mortalities of 100%, but at 1% the mortalities were 97% to 98%

6. Bed Bugs. Research at Orlando, Fla., to discover and develop new insecticides for the control of bed bugs was continued. Forty-one candidate compounds were screened against bed bugs by exposing them to residues of the insecticides on filter paper (50 mg. per square foot). Of these compounds, 12--Bayer 29493, Bayer 30237, Bayer 30554, Bayer 29492, Bayer 41831, carbo-phenothion, Ciodrin, Dicapthon, EPN, Hercules AC-5727, Methyl Trithion, and Stauffer N-2404--were equal to or better than the standard, malathion, since they caused 95% to 100% mortality of bed bugs when bed bugs were exposed for 24 hours to residues that had aged 8 to 12 weeks.

Research has been conducted to develop standardized techniques for evaluating the presence and degree of resistance to insecticides in bed bugs. Studies with malathion and Bayer 29493 have determined the concentrations and exposure times to be used with these two insecticides to allow detection of susceptible and resistant strains of bed bugs.

7. Ants. Mirex bait, a formulation containing 0.25% mirex and peanut butter, was evaluated for its effectiveness in controlling a natural infestation of Pharaoh ants in insectary rooms of the Orlando, Fla., laboratory. The bait, placed in feeding stations constructed of small plastic lids covered with screen that permitted entry of the ants, eliminated the ants from the insectary rooms.

C. Biological Control

1. Mosquitoes. At Fresno, Calif., in cooperation with the Bureau of Vector Control, California State Department of Health, studies were initiated on biological control agents for mosquitoes. Studies have been initiated on the host, parasite, and host-parasite relationship of mosquitoes and various species of microsporidia of the genus, Thelohania.

D. Insect Sterility, Attractants, and Other New Approaches to Control

1. Mosquitoes. Extensive studies on the chemosterilization of mosquitoes were continued at Orlando, Fla. In laboratory tests, none of 84 compounds evaluated as larval sterilants against Aedes aegypti were as effective as apholate which consistently gave 94-100% sterility, but 5 compounds reduced egg hatch to 20% or less. Various combinations of apholate and tepa were no more effective as larval sterilants than either chemical alone. Granular formulations of apholate (pyrophyllite granules, corncob grits, buffered vermiculite granules) were slightly less effective than solutions of apholate in sterilizing A. aegypti larvae. When tested in the presence of soil, the sterilant activity of granular formulations and solutions of apholate was severely diminished. Formulations of apholate on pyrophyllite dust (which floats on water surface) gave consistently higher sterility in A. aegypti larvae than granular formulations under various test conditions including the presence of soil. At sterilizing concentrations tepa was more toxic to A. aegypti and Anopheles quadrimaculatus larvae than apholate. All instars of A. aegypti larvae were affected by treatment with tepa, but those exposed from the third instar to pupation had the highest degree of sterility. Under laboratory conditions, tepa sterilized A. aegypti larvae for at least 6 days. Under field conditions, tepa was only partially effective and had no sterilizing effect after 3 days.

Pupae of Anopheles quadrimaculatus exposed to tepa were killed or failed to emerge at high concentrations. At lower concentrations, some individuals survived, but were not sterilized. Males of Aedes aegypti sterilized as larvae by treatment with apholate were capable of competing with normal males in mating normal females. In these tests individual females mated with both sterilized and normal males. With A. quadrimaculatus residual deposits as low as 0.34 mg./sq. ft. of glass surface completely sterilized adult females; males were not tested.

Females of Aedes aegypti sterilized completely by contact with residues of tepa did not transfer sterility to males during copulation. Males of

Anopheles quadrimaculatus treated with radiation at a level that did not induce complete sterility and by chemicals which caused complete or almost complete sterility competed equally with normal males for mating normal females. Both sexes of adult Aedes taeniorhynchus were rendered sterile by exposure to residual deposits of tepa and metepa.

In Oregon tepa and ENT 50450 were tested as sterilants against Culex tarsalis adults by spraying in wind tunnel tests, and against larvae by exposure in water. Tepa sterilized males and females at a spray concentration of 6%; ENT-50450 sterilized only males at this concentration. Lower concentrations of either compound were not effective. Against larvae, both compounds were toxic at higher test concentrations and ineffective and partial toxicants at lower concentrations.

In Florida 124 compounds were screened against mosquitoes as personal-use or clothing repellents and 236 for space repellency. None of those screened as space repellents showed a high degree of repellency. Five compounds screened as personal-use or clothing repellents showed sufficient promise for further testing. Storage tests were made with wrapped and unwrapped Army fatigue trousers and jackets. Garments treated with M-1960 and deet gave complete protection against Aedes aegypti after 6 months in storage. However, one week after removal from storage effectiveness was reduced. There was little difference in the effectiveness of deet as a skin repellent against A. aegypti when the deet was applied at similar concentrations as a pressurized foam, a zinc oxide ointment, or an ethanol solution. Deet stored in a tin can since November 1954 was as effective as a sample received in 1961 in tests with skin applications against A. aegypti.

In Oregon studies have shown that grass infusion and log pond waters are attractive to ovipositing female Culex pipiens quinquefasciatus. Distilled water treated with methane or furfural was more attractive to ovipositing females than distilled water, but less attractive than log pond water.

2. House Flies. Research on the development of sterilization for the control or eradication of house flies has been continued. In Florida, investigations involved the screening of candidate chemosterilants, secondary laboratory tests with promising compounds, experiments related to actual field conditions, experiments with natural populations, and fundamental studies on effects of chemosterilants on house fly physiology and cytology. Eight hundred and eighty-six new chemicals were screened for sterilant activity. Of these some produced toxic effects; however, 90 caused complete or partial sterility in the treated house flies. Many promising compounds have been further evaluated for their effects on individual sexes, their maximum tolerated as well as minimum effective dosage, and other methods of administering the compounds to house flies. Seven compounds were highly effective in sterilizing both sexes of house flies, but two of these were also toxic at the dosages tested. Metepa and tepa as residual deposits on glass sterilized house flies at dosages from 25 to 250 mg. per sq. ft., but 5-fluorourotic acid was ineffective as a residual deposit at similar dosages. Apholate

in the adult food sterilized house flies regardless of their age and the flies did not regain fertility. Motile sperm were present in the testes of chemosterilized males and transferred during copulation to the spermathecae of females throughout the life span of the male.

Tests were initiated to determine whether a dosage of a chemosterilant too low to prevent hatching or adult emergence might by the accumulation of small genetic injuries eventually reduce or eliminate reproduction. With apholate, one colony showed reduction of oviposition in the 4th, 5th, 6th, and 7th generations and no individual reached the pupal stage in the 7th generation. With metepa, the 5th through 9th generation of another colony showed reduced oviposition and the 10th generation failed to oviposit.

Preliminary tests with apholate, tepa, and metepa on house flies indicated that the probit of the percent sterility with house flies can be related to the log of the concentration. Metepa and apholate shortened the life span of adult house flies considerably, but 90% or more of the male population survived the first 10 days, or that period of time in which mating activity is the greatest. Survival during the first 10 days was essentially the same in treated and nontreated house flies. In other experiments house fly pupae were dipped in solutions of tepa, apholate, or metepa. This treatment was toxic to most individuals, but a high degree of sterility occurred in individuals surviving the treatment.

Tests have been conducted with various formulations and types of baits for administering chemosterilants to house flies. Effective formulations of both dry baits and paint-on liquid baits have been developed.

A method has been devised for studying chromosomes of house flies. Using this technique, the effect of chemosterilants on spermatogenesis and oogenesis will be made.

Chemosterilant baits have been applied in privies on two islands (metepa on Grand Turk and apholate on Mayaguana) in the Atlantic Missile Range. On a third island (San Salvador), Dipterex bait was applied to provide a comparison between an insecticide and the chemosterilants. House fly breeding was limited to privies, almost exclusively, but the vast majority of feeding by house flies occurred in the kitchens. The baits were applied weekly at first, and then semiweekly. After several months the dry bait formulation was changed to a heavy sugar syrup which was sprayed in the privy pits. The metepa treatment on Grand Turk caused a high degree of sterility in eggs deposited by female house flies (usually 65% to 80% with dry baits and 80% to 86% with liquid baits). The total sterility may well have exceeded 95% since a high proportion of females did not lay eggs. The number of flies decreased gradually after treatment. There was a marked reduction in house fly populations, but complete eradication was not achieved after several months of treatment. Auxiliary methods of control have been initiated in an attempt to achieve complete eradication on this island. In other localities, where the principal feeding by house flies occurs outside houses in

places where baits might be applied, the bait alone might be sufficient. The dry bait treatments of apholate on Mayaguana were less effective than those of metepa on Grand Turk. This difference was attributed to the type of breeding places on the two islands. The abundance of flies was never high on San Salvador. Before treatment, grid counts ranged from 2 to 3 and after treatment they were less than 1.

In laboratory tests with the little house fly at Corvallis, Oreg., tepa and metepa administered either in the adult or as residual deposits sterilized surviving adult flies but were highly toxic at the concentrations or dosages used.

In tests in Oregon with an olfactometer and with simulated, treated fly models ("Pseudo flies"), the presence in female house flies of a volatile chemical or chemicals that can influence the behavior of male house flies was demonstrated. The behavior modification was in the nature of attraction to the source of the pheromone or an excitation of mating behavior patterns. The material, which was benzene soluble and relatively stable, was sex related and specific to the house fly.

3. Cockroaches. In Florida further tests were conducted with chemicals in the food or nymphal German cockroaches to determine their possibilities as sterilants. Apholate (2%) and metepa (1%) caused delayed development and were toxicants. Kepone (0.01%), aphomide (2%) and NSC-18016 (1%) caused delayed development and were partial toxicants. Methiotepa (0.25%) and methyl apholate (1%) caused delayed development in the nymphs, but methiotepa at 0.1% had no effect. Methiotepa and methyl apholate caused complete or reduced sterility in either sex of cockroaches and complete sterility when both sexes were treated.

4. Eye Gnats. Hippelates pusio can be sterilized with either apholate or tepa. However, in some cases mortality was high or the treatments were only partially effective. When chemosterilants were fed to adult gnats, tepa killed all or nearly all of the gnats at the 0.5% concentration. It also killed 58% of the gnats in the initial exposure at 0.1% but was not toxic thereafter or at lower dosages of 0.05% and 0.01%. Every concentration of tepa which was tested produced complete sterility when freshly prepared food was used. Apholate was much less toxic than tepa when fed to adult gnats. This compound sterilized all or nearly all of the gnats at concentrations of 0.5% and 0.1% in the adult food, caused some sterility at 0.05%, but was ineffective at 0.01%. In tests in which gnats were allowed to walk on residual deposits, tepa caused sterility at dosages as low as 3 mg./sq. ft. Apholate residues of 5 mg. or 10 mg./sq. ft. did not cause sterility in gnats. This work was conducted at Orlando, Fla.

5. Body Lice. At Orlando, Fla., tests were run to find chemicals that have systemic insecticidal or repellent action. In these tests candidate compounds were administered to rabbits orally by stomach tube or in their food, and body lice allowed to feed on the rabbits at various time intervals after

treatment. Of 63 compounds tested at various dosages (mg./kg.), 8 compounds caused complete mortality of lice without affecting the rabbits. The compounds and the lowest effective dosages were: Bayer 30468, 10 mg./kg.; Bayer 33904, 50 mg./kg.; Monsanto CP-19203, 10 mg./kg.; American Cyanamid CL-38064, 25 mg./kg.; Tandearil, 10 mg./kg.; Hercules AC-3273, 200 mg./kg.; American Cyanamid CL-26691, 10 mg./kg.; and American Cyanamid 18706, 100 mg./kg. Other compounds caused some or complete mortality of lice but affected or killed the rabbits at dosages which were effective for the lice. These compounds were Stauffer N-3422, American Cyanamid CL-38106, Monsanto CP-12432, heptachlor, and O, O-dimethyl 1-acetoxyethyl-2,2,2-trichlorophosphate. Of 8 antibiotics tested for systemic activity against body lice, and butazolidin for systemic activity against the ear mite of rabbits, none were effective in killing lice or mites. A dose of 75 mg./kg. of Shell 52-RL-71 administered by stomach tube to rabbits caused 100% mortality of lice from 2-21 days after treatment.

6. Mites, Ticks, and Fleas. Research was continued in Florida on the development of repellents and other methods for protecting humans from mites, ticks, and fleas. One compound--Shell 52-RL-71 which had shown a long period of effectiveness as an oral systemic in rabbits against both body lice and Aedes aegypti mosquitoes--and 24 other selected compounds were tested as oral systemics in guinea pigs against oriental rat fleas. Shell 52-RL-71 gave an extended residual effect similar to that found with body lice and mosquitoes. Of the 24 other materials, 11 compounds caused complete mortality of some lots of fleas within 5 hours after treatment without noticeably affecting the guinea pigs. Four compounds caused complete mortality of some lots of fleas within 5 hours after treatment but seriously affected or killed the guinea pigs.

Screening tests to find new repellents for application to clothing utilizing nymphal lone star ticks or chigger mites as the test species were continued. Of 109 new compounds tested against the nymphal lone star tick, 17 were rated as Class IV materials giving 80% repellency for periods ranging up to 50 days. Against chiggers none of 147 materials screened were effective enough to be rated Class IV materials.

With chigger mites the effect of aging on the effectiveness of deet impregnated in cloth was determined using 5 concentrations of deet. At 1.0 and 0.75 gms./sq. ft. deet remained effective for 6 days, at 0.5 and 0.25 grams for 5 days, and at 0.125 grams for 4 days.

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ANALYSIS, SYNTHESIS, FORMULATION, AND EVALUATION OF
INSECT CONTROL CHEMICALS

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Problem. Modern insecticides are at present the most rapid and effective means of controlling injurious insects and their use has enabled the American farmer to produce an abundance of high quality crops and livestock. This extensive use, however, has been accompanied by increasing resistance of some insects to certain insecticides and by the possibility of leaving harmful residues on or in harvested crops, in meat, or in dairy and poultry products. There is therefore a need for the development of new types of chemicals, from natural sources and through synthesis, to which insects will not become resistant. These chemicals should be safe to handle and not leave harmful residues in the harvested products used for foods or feeds, or adversely affect wildlife, beneficial insects and other desirable organisms. More effective formulations of chemicals should be developed for the control of different insect species under various environmental conditions. Such chemicals and formulations require initial testing in the laboratory and evaluation under field conditions before they can be recommended for practical use. It is essential that accurate, sensitive analytical methods be developed for the determination of the amounts of chemicals deposited and the rate of disappearance of their residues and breakdown products in treated crops, animals, or soils. Better attractants are needed for use in traps and bait sprays for both insect detection and control. Research also is needed on repellents that would be useful in controlling insect attacks on crops, livestock, and man.

USDA PROGRAM

The Department has a long-term program supported by chemists, entomologists and scientists of other specialized disciplines to discover and develop new and improved insect control chemicals and methods of applying them. Chemical research to discover, isolate and identify products of natural origin which can be employed for insect control is carried on mainly at Beltsville, Md.; components of the cotton plant that serve as attractants or essential nutrients, or otherwise affect the boll weevil are being investigated at State College, Miss., in cooperation with the Mississippi Agricultural Experiment Station. Chemical research on synthetic organic materials and formulations for insect control is carried out at Beltsville, Md.; Gainesville, Fla.; and State College, Miss. Development of analytical methods for insecticide residues is carried on at Beltsville, Md. (headquarters); Tifton, Ga.; Vincennes, Ind.; Kerrville, Tex.; and Yakima, Wash. There is cooperation with the State Experiment Stations in the respective regions of all these laboratories. Cooperative work with the States on insecticide residues is carried on in connection with the following Regional Research Projects: NC-19, Fundamental Problems Associated with the Accumulation of Pesticidal Chemicals in Soils; NC-33, Pesticide Residues on or in Food, Feed, and Forage Crops; NE-36, Determination of Pesticide Residues on Raw Agricultural Commodities; S-22, Pesticide Residues on Plant and Animal Products and Soils; and W-45, Pesticide Residues, Their Nature, Distribution, and Persistence in Plants, Animals, and

Soils. Research on aerosols for insect control is conducted at Beltsville, Md. Biological evaluation of insecticides and other types of insect control chemicals is carried on at Beltsville, Md., and Brownsville, Tex. Research on methods for control of insects in aircraft is done at Beltsville, Md.

The Federal scientific effort devoted to research in this area totals 39.0 professional man years. Of this number 6.0 is devoted to products of natural origin as sources of insect control materials; 16.0 to development of synthetic organic materials and formulations for insect control; 5.0 to methods of analysis for insecticide residues; 2.0 to aerosols for insect control; 7.0 to biological evaluation of insect control chemicals; 1.0 to methods for control of insects in aircraft; and 2.0 to program leadership.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Products of Natural Origin as Sources of Insect Control Materials

1. Insect Sex Attractants. In the research carried on at Beltsville, Md., on natural insect sex attractants, the attractant produced by virgin female American cockroaches has been isolated and identified as 2,2-dimethyl-3-isopropylidenecyclopropyl propionate. This substance elicits a response from the males of the species in amounts less than 10^{-14} microgram. Attempts to synthesize the compound are in progress. Its structure is of a very unusual type which presents great difficulties in the synthesis.

In an investigation of the sex attractant of the pink bollworm, an extraction and chromatographic procedure has been developed at Beltsville which permits extraction of the whole bodies of the female bollworm moths, thus avoiding the time and labor required to clip off the abdomens of the moths.

In work with gyplure, the synthetic sex attractant for male gypsy moths that was discovered at the Beltsville laboratory, it has been found that some commercial batches may contain as many as 10 different constituents (byproducts and other impurities in addition to gyplure itself), whereas laboratory-prepared samples contain only 3 or 4. Methods have been developed for separating these different materials and determining the amount of pure gyplure in samples. The degree of attractancy depends on the purity, since the presence of more than small proportions of certain of the impurities results in inactivation of the attractant effect of the gyplure.

As an aid to the elucidation of chemical structures, a hydrogenolytic chromatographic procedure was devised for the interpretation of molecular configuration, using only a few micrograms of material. Called "carbon-skeleton chromatography," it has aroused great interest among the scientific fraternity. It proved very useful in establishing the structure of the American cockroach sex attractant.

2. Materials of Plant Origin for Insect Control. A material known as trans-Asarone that was isolated from Indian calamus oil at Beltsville has shown toxicity to adult body lice, Aedes mosquito larvae, and adult

mosquitoes in laboratory tests made at Orlando, Fla.

A sample of an extract of ground-up stems of Anona glabra (a wild plant in Florida) prepared at Beltsville for insect tests was sent to the National Institutes of Health, where it showed antitumor activity. Preliminary results of tests with different fractions of the extract indicated that the alkaloid fraction had the greatest antitumor effect.

At State College, Miss., a chemical investigation is in progress on the purification and identification of substances in the cotton plant that attract, arrest, or repel boll weevils.

B. Development of Synthetic Organic Materials and Formulations for Insect Control

1. Preparation of Synthetic Organic Compounds for Testing as Insect Control Agents. The research at Beltsville, Md., and Orlando, Fla., on insect chemosterilants, chemicals capable of suppressing the reproductive capacity of insects, is yielding promising results. The compounds that have been most consistently effective in inhibiting the reproduction of insects are the derivatives of aziridine (ethylenimine) and therefore they have received the greatest attention. A large number of new aziridines, both N-substituted and C-substituted, have been synthesized at Beltsville for evaluation at various field stations of the Division. In tests at Beltsville, apholate was an effective sterlant for male American cockroaches. A new compound of the dicarboxamide class that was synthesized was effective in sterilizing screw-worms in laboratory tests. A small quantity of radioactive tepa was synthesized for use in metabolism studies at Beltsville. At State College, Miss., the effect of some chemosterilants on the boll weevil is being studied.

Because of the importance of the aziridines, intensive studies have been made of their physical and chemical properties and new assay methods have been devised involving colorimetric tests, thiosulfate titration, paper chromatography, thin layer chromatography, and nuclear magnetic resonance. The last-named method is being used to investigate the stability of aziridines under various conditions of temperature and acidity. Since these compounds are unusually sensitive to acids, their sterilizing activity is dependent on the pH of the media, and careful attention must be given to their formulation. One method of formulation found useful for water-insoluble derivatives is emulsification with hydroxylecithin.

A special device has been constructed to separate male and female fruit flies (Drosophila) for chemosterilant studies. It uses a constant-velocity air stream to lift the lighter males into a separate chamber.

In cooperating with the Plant Pest Control Division operation to rid Florida of the Mediterranean fruit fly, a third isomer of the medfly attractant trimedlure was discovered. This isomer, which is completely unattractive to the medfly, comprised more than one-third of some lots of

the commercial lure purchased to detect the pest. A gas chromatographic procedure was developed for assaying the three isomers in trimedlure and a new purchase description was written to insure that the Plant Pest Control Division will obtain a superior lure in the future.

Considerable effort is being devoted to attempts to synthesize the American cockroach sex attractant referred to under heading A. The extremely unusual structure of this attractant molecule does not readily lend itself to synthesis. The dihydro analog of the attractant has been made, but it has no biological activity.

A number of new compounds were especially prepared as candidate attractants. Efforts were largely focused upon attractants for the European chafer, Mexican fruit fly, and medfly.

2. Formulations. Research on the development of improved formulations of insect control materials were conducted at Beltsville, Md., and Orlando, Fla. Two formulations were developed for the production of stable emulsions of the insect repellent deet, which is difficult to emulsify, especially in hard water. Simple procedures were devised for assessing emulsion stability.

At the request of the U. S. Army a proposed Federal Specification was drafted for an emulsifiable concentrate of deet suitable for field use to impregnate clothing and uniforms.

Gas chromatographic procedures have been devised for the analysis of two insect repellents, ethylhexanediol and deet.

Cooperation was extended to several national and international agencies interested in the improvement of pesticide dusts and powders. A nonwettability test was developed and proposed as an addition to the Federal Specification for 10% DDT powder. Three methods for evaluating the compactibility and flowability of dusting powders were reviewed and submitted to the Association of Official Agricultural Chemists. This study was also of interest to insecticide producers and to the World Health Organization. At the request of the Crops Research Division, the draft of a proposed specification for simazine herbicide dispersible powder and granular formulation was reviewed. Assistance was given to the U. S. Army Quartermaster in the development of a new type of oil-impregnated granules. At the request of General Services Administration the Interim Federal Specification for granulated aldrin was redrafted for conversion to regular Federal Status. The Interim Federal Specification for DDT water-dispersible powder underwent a similar revision. Physical tests were performed for the Plant Pest Control Division on the mirex bait used to control fire ants. Assistance was given to the U. S. Navy in connection with a DDT dusting powder having excessive wettability. In cooperation with the States of Oregon and Washington and the U. S. Forest Service a number of DDT-diesel oil samples were analyzed for conformance with specifications. Specimens of petroleum coke, wheat bran and corn cobs were evaluated as candidate

diluents. Polyethylene was found to improve the persistence of DDT and heptachlor sprays. It was shown that emulsified tung oil was suitable for the preparation of Sevin sprays to control gypsy moths.

3. Testing of Respiratory Protective Devices. Tests were conducted at Beltsville with respirators available commercially for protection against pesticides. A study was made of components used in filters and cartridges for respiratory protective devices. It was found that activated charcoal made from coconut shells is more efficient than charcoal made from petroleum residues. Filter masses made from a mixture of fibers are more efficient than those in which one type of fiber is used alone. An ARS publication and later a supplement to it were issued on protective respiratory devices. Copies have been in great demand from pesticide operators, growers, agricultural extension workers, the pesticide industry, public health workers, and others.

C. Methods of Analysis for Insecticide Residues

A method of analysis has been developed for the determination of residues of General Chemical 4072 in the fat and other body tissues of cattle. The method comprises extraction and clean-up of the sample and hydrolysis of the General Chemical 4072 with 12N sulfuric acid to produce 2,4-dichlorophenyl chloromethyl ketone, followed by gas chromatography of a hexane solution of the hydrolysis product.

An analytical method for dimethoate residues described in last year's report has been modified and adapted to the analysis of dimethoate in milk.

A colorimetric method that was developed in 1960 for the determination of coumaphos (Co-Ral) residues in animal tissues has been modified to make it applicable to the determination of coumaphos in dip solutions from animal-dipping vats. A fluorescence method has been devised for determining the presence of coumaphos on cattle hair; this provides a quick, easy means of determining whether cattle passing through screw-worm quarantine lines have been dipped in coumaphos.

A method was developed for determining ronnel in animal-dip solutions and on the hair of animals that have been dipped. The method involves hydrolysis of the ronnel to liberate 2,4,5-trichlorophenol and determination of the latter by ultraviolet spectroscopy.

The sulfide method of analysis for dioxathion (Delnav) has been modified to adapt it to the analysis of dips.

In cooperation with the Poultry Research Branch of the Animal Husbandry Research Division research has been continued to develop analytical methods for terephthalic acid (TPA) residues in chicken tissues. This acid is a synergist for certain antibiotics in treating chronic respiratory disease of poultry. A fluorometric method has been developed for this purpose and

and some data obtained on TPA residues in tissues of chickens fed a TPA-treated diet.

Another continuing emergency investigation being carried out at the request of the Pesticides Regulation Division concerns methods of analysis for chloroanilines produced by hydrolysis of detergents containing trichlorocarbanilide (TCC). Cases of cyanosis had been reported in premature babies wearing diapers that were laundered with TCC-containing products and autoclaved. Methods of analysis have been developed and it has been shown that chloroanilines are produced when solutions of certain detergents containing TCC are boiled. Diapers treated with a rinse additive containing TCC and then autoclaved contained about 35 parts per million of chloroanilines.

D. Aerosols for Insect Control

A U. S. public service patent (3,088,680) has been obtained for a dispenser for aerosols or similar pressurized products so designed that it will discharge the liquid contents when the container is held in any position. The usual aerosol dispensers will discharge the liquid only when the container is held upright. The new device is now being used on all pressurized ethylene oxide sterilizer units purchased by the Armed Forces.

A compact unit has been designed and constructed for testing the flammability of liquefied-gas-propelled aerosol formulations. Flammability tests have been made of a number of representative insecticide aerosol formulations of types that are frequently proposed to the Pesticides Regulation Division for label approval and registration and the results have been reported to that Division.

At the request of the World Health Organization tentative specifications have been drafted for household-type liquefied-gas-propelled insecticide aerosols and also for mist machines and thermal aerosol machines.

In cooperation with the aerosol industry a new lot of the Official Test Aerosol (OTA) formulation has been prepared and packaged for use during 1963-1965 as a standard for insecticide aerosol evaluation.

The stability of some organic phosphate insecticide aerosols has been investigated. An aerosol containing TEPP in methyl chloride, which was prepared in 1947, has recently been examined. Although the aerosol solution contained a white precipitate, it was still effective in killing aphids. DDVP in methyl chloride showed no loss in effectiveness over a 6-month period. DDVP-containing aerosol formulations will severely corrode aluminum containers but have little or no effect on tin-coated containers if the moisture content is low.

A small unit has been designed for sterilizing insect-rearing medium with a pressurized ethylene oxide sterilant formulation. The unit will hold six 4-inch petri dishes containing the medium.

Tests were made with various insecticide aerosol formulations dispersed by a thermal aerosol machine in greenhouses. The machine was a commercial Dynafog Model 70 that had been modified for use with small quantities of formulations. Applications of 1 gram of DDVP in 10 grams of methyl chloride per 1,000 cu. ft. by this means was very effective against mites and aphids.

A pressurized tree-injection apparatus has been developed in cooperation with the Crops Research Division. An isobutane-propane propellant mixture operates well. The back pressure in this type of treatment requires a special valve.

E. Biological Evaluation of Chemicals for Insect Control

1. Insecticides. A major activity in this area is the laboratory testing of synthetic organic compounds and natural products against representative species of insects to determine whether the materials have insecticidal, synergistic, attractant, repellent, insect chemosterilant, growth controlling, or other effects that would be useful for insect control. Preliminary evaluation tests on these materials are carried out at Beltsville, Md., and Brownsville, Tex., by the Pesticide Chemicals Research Branch and at 26 other locations by other Branches of the Division, throughout the United States and in Mexico, on 61 insect species and 9 mite species. Materials that show evidence of effectiveness are distributed to laboratories of the Division for more extensive secondary testing of their value. Some of the materials tested originate within the Pesticide Chemicals Research Branch and many others are supplied by other government or private research agencies and by industry.

Eight hundred eighty-five compounds and extracts or fractions of natural products were distributed for preliminary screening tests as insecticides during the year. The most effective of these materials were then distributed for secondary testing. Sixty-eight of the materials showed high toxicity to at least one species of insect or mite in laboratory tests.

A cooperative study is being made with the Insecticide Scientific Committee of the Chemical Specialties Manufacturers Association to develop a method for the evaluation of residual sprays against German cockroaches. Chlor-dane residues of 33 mg./sq. ft. deposited from a kerosene spray on glass and Masonite surfaces have given almost complete control of nonresistant roaches for 5 weeks.

2. Materials that Control the Activities of Insects Through Effects Other than Death. Increased emphasis has been given to the search for materials of synthetic or natural origin with properties that could be used to control insects by effects other than kill. One phase of this research is the investigation of insect sex attractants that are useful for detection of infestations and might be used for control by luring insect pests into traps or into contact with toxicants or sex sterilants.

Researchers at Beltsville developed a method for collecting and concentrating the sex attractant of the virgin female American cockroach. The crude attractant thus obtained was purified by the chemists and its chemical structure determined, as described under heading A. The course of the chemical work was guided by biological tests of the attractant activity of the various fractions obtained.

The female German cockroach also produces a sex attractant. The male German cockroach does not react as vigorously as does the American cockroach to its sex attractant and a different type of assay procedure therefore is being investigated.

Investigation of the sex attractant produced by the female southern armyworm moth has been continued. The insects are being reared at the Brownsville, Tex., laboratory and extracts prepared from the females for work on the isolation and identification of the attractant by the Beltsville chemists.

Large scale rearing of *Cecropia* moths is in progress at Beltsville to provide a source of insect juvenile hormone. This hormone prevents the development of insects to the mature stage and might have possibilities for control of insect pests. The material obtained from the moths is for use in a chemical investigation of the nature and composition of the hormone.

In connection with research on insect chemosterilants to prevent insect reproduction, a study has been made of the influence of pH on the effect of tepa (tris(1-aziridinyl)phosphine oxide) on fertility of house flies. The sterilizing effect on male flies was several times greater when the tepa was used in injection tests in alkaline solution than in acid. A laboratory study is in progress of the comparative effectiveness of tepa and apholate as chemosterilants for male house flies.

3. Aerosols and Space Sprays. Tests have been carried out with formulations of several of the newer insecticides in aerosol or space spray form to evaluate their effectiveness against resistant and nonresistant flies or mosquitoes. In connection with the preparation of the new lot of Official Test Aerosol for 1963-1965, referred to under heading D, biological tests were made to compare its effectiveness with the previous lot of this standard.

Several commercially available synergists for pyrethrins were compared for effectiveness in space sprays against resistant house flies. Sulfoxide was as effective as piperonyl butoxide, but propyl isome and MGK-264 were less effective. Sesame solids extracted from the seeds of the Northern Rhodesian black sesame plant, Sesamum angolense, synergized pyrethrins about two-thirds as well as did piperonyl butoxide against susceptible house flies.

Ten different carbamate insecticides were tested alone and in combination with piperonyl butoxide in space sprays against house flies in the laboratory. With two of these piperonyl butoxide showed a synergistic effect, with three an antagonistic effect, and with the remainder there was no marked degree of either synergism or antagonism.

F. Methods for Control of Insects in Aircraft

The method of aircraft treatment with DDT micronized dust to prevent the spread of Japanese beetles has been used by the Plant Pest Control Division for the past year in connection with Japanese beetle quarantine. Recent tests have indicated that a dosage of one gram of the 85% DDT dust per 1,000 cubic feet could be used satisfactorily instead of the two-gram dosage now in use.

Further cooperative tests have been carried out with the World Health Organization on the aerosol treatment of aircraft at "blocks away" (just after the door is closed for departure). Tests were conducted on airline flights in the Philippines and the Fiji Islands. The WHO standard reference aerosol containing pyrethrins and DDT with xylene and odorless petroleum distillate as solvents was compared with formulation G-1492, prepared by USDA and containing a higher concentration of pyrethrins with DDT and xylene. Both formulations were highly effective against nonresistant mosquitoes, but G-1492 was considerably more effective against resistant mosquitoes. G-1492 caused a little nasal irritation in passengers; it has been recommended, however, that this formulation be used where the presence of resistant insect strains has been proved.

Tests have been made with a pressurized formulation of ethylene oxide and propellents 11 and 12 against American cockroaches, confused flour beetles, resistant and nonresistant house flies, mosquitoes (Aedes aegypti), Japanese beetles, and grasshoppers. A dosage of $\frac{1}{4}$ lb. of ethylene oxide per 1,000 cu. ft. for 18 hours, 2 lb. for 3 hours, or 4 lb. for 2 hours killed 100% of adults and eggs of all the insects except the confused flour beetle, which required more than 48 hours. This is about 1/20 the dosage required to kill 100% of bacteria and other microorganisms.

In connection with some proposed experiments on the effects of radiation in space on insects, tests have been made on the irradiation of silkworm eggs with gamma rays. Eggs just out of diapause were much more susceptible to damage by the radiation than those in a later stage of development.

The effect from split radiation dosages was less than from the same total dosages given at one time. An insect-egg holder has been designed that utilizes photographic emulsion plates to permit the easy determination of heavy particle cosmic ray hits.

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IDENTIFICATION OF INSECTS AND RELATED ARTHROPODS
Entomology Research Division, ARS

Problem. Only about a third of the estimated two million or more kinds of insects in the world have been described and named. Many of these are of no immediate concern to agriculture or mankind, but thousands of species are potentially destructive or useful. Minute morphological differences are very important in recognizing many species, and only highly trained specialists are able to positively identify known species and describe new ones. Precise information on the identity and distribution of insects is essential to the efficient conduct of programs concerned with research on harmful insects and the development of methods for their control, and in the management of regulatory activities intended to exclude, control, or eradicate insect pests.

Knowledge of the classification and identification of insects at present is far from adequate. Knowledge of the insect fauna of the world provides the best assurance that any potential pests will be immediately recognized, so that appropriate safeguards can be set up to exclude them or prompt action taken to control or eradicate them if accidentally introduced. Moreover, with increasing emphasis on the utilization of beneficial insect parasites and predators to help control destructive insects, it is necessary that we know which insects to search for, where they might be found, and how to recognize those that may be useful.

USDA PROGRAM

This program of the Department is a long-continuing one involving insect taxonomists, and includes basic research to make known to science previously unrecognized and undescribed species of insects, ticks and mites, and the application of results of this research to the problem of insect identification. The work is carried on to a limited extent at Beltsville, Md., but mostly in Washington, D. C., in close cooperation with the U. S. National Museum of the Smithsonian Institution. Somewhat less active cooperation is maintained with various centers of taxonomic research in the United States and foreign countries and with numerous individuals in many parts of the world.

The Federal scientific effort devoted to research in this area totals 30.0 professional man-years. Of this number, 6.8 is devoted to basic studies to name and describe beneficial and injurious insects, mites, and ticks; 15.4 to identification of insects, mites, and ticks; 6.8 to preparation of keys and monographs on the classification, distribution, morphology, and biology of insects and related arthropods; and 1.0 to program leadership.

Research in this area is also conducted under three P. L. 480 Projects. S9-ENT-6 (Uruguay) provides 1.5 professional man-years, F4-ENT-2 (Egypt) provides 4 professional man-years and A7-ENT-24 (India) provides 3 profes-

sional man-years. The Uruguay project is concerned with the classification of grasshoppers; the Egypt project with a survey of Egyptian insect fauna; and the India project (started in April 1963) with a systematic study of thrips. 35-ENT-2 (Colombia) has 2 man-years on Drosophila classification. A7-ENT-17 (Pakistan) provides 1.5 man-years on leafhopper taxonomy.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Basic Studies to Name and Describe Beneficial and Injurious Insects, Mites, and Ticks

1. Whiteflies. Whiteflies of the genus Trialeurodes are important economic pests and at least one species has been implicated as a vector of the yellow dwarf virus of sweetpotatoes. A list showing the numerous wild and cultivated host plants and the distribution of 5 of the most important species of the genus has been made available.
2. Biting Midges. For many years the most abundant and annoying biting midge in the mountains and woodlands of Northeastern United States and Canada has been known as Culicoides obsoletus. It has now been determined that there are 4 distinct species in eastern North America. These have been described and their known distribution and breeding habits reported.
3. Tetrastichus spp. Tetrastichus is one of the largest and most diverse genera in the Chalcidoidea. Many species are primary parasites, others are secondaries. Ten new species have been described adding considerably to the knowledge of this complex group.
4. Leafhoppers. Many species of leafhoppers are of great economic importance because they are vectors of plant diseases. Six new species and a new genus have been described from Central and South America.
5. Diptera Catalogue. A synoptic catalogue of the Diptera of America north of Mexico has been completed. It will be an indispensable aid to taxonomists working on Diptera just as the Hymenoptera Catalogue has been for specialists on that order. Preparation of the catalogue has taken a high proportion of the time of the dipterists working in this area.

B. Identification of Insects, Mites, and Ticks

Authoritative identifications and references to pertinent taxonomic and biological literature are supplied in support of Federal and State research, control, and regulatory activities pertaining to entomological problems. These services are also performed for industry, pest control operators, and private individuals in the United States and for foreign agencies and institutions concerned with entomology.

During the year, a total of 33,475 lots of insect material was received for identification. Well over 316,000 specimens were examined. A total of

84,377 identifications were made and reported. Material was not accepted for identification unless appropriate specialists were available to examine it or unless there was justification for the identifications. Nevertheless, the backlog of material awaiting study and report increased considerably.

The sources of material and the numbers of identifications made for each are shown in the following table:

<u>Source</u>	<u>Number of Identifications</u>	<u>Percent of Total</u>
Agricultural Research Service		
Plant Quarantine Division	22,095	26.19
Plant Pest Control Division	5,126	6.08
Entomology Research Division	4,107	4.87
Forest Service	1,544	1.83
Agricultural Marketing Service	870	1.03
Other Federal Agencies	1,953	2.31
States and Insular Possessions	21,532	25.52
U. S. individuals	21,520	25.50
Foreign agencies and individuals	<u>5,630</u>	<u>6.67</u>
Total	84,377	100.00

Many specimens received for identification represented species not previously in the National Collection, or they documented new distributional data. For these reasons 68,873 specimens were added to the Collection during the year.

The systematic review of technical literature essential to the programs in this area included the examination of 1,775 publications which contained 5,064 articles of scientific interest. A total of 2,031 articles was catalogued. From these 6,906 index cards to authors were prepared, and 15,498 cards were made up on which data of significance to taxonomists were recorded.

During the year 101 visitors obtained aid on taxonomic or nomenclatural problems. Some visitors remained only an hour or so, but many stayed one or two days and 8 remained for more than a week.

C. Preparation of Keys and Monographs on the Classification, Distribution, Morphology and Biology of Insects and Related Arthropods

1. Mites. The importance of phytophagous and predaceous mites as agricultural pests, carriers of diseases and as biological control agents can hardly be overemphasized. Several publications adding to available knowledge of their classification have been completed.

2. Moths. A revision of the family Cosmopterygidae and descriptions of new species of Perimede have added greatly to the knowledge of these tiny moths, many of which are leaf miners and of economic importance.

3. Two-winged Flies. An annotated list of Genus-Group names in the Family Simuliidae has been published. These black flies, or buffalo gnats, are vicious biters and serious pests. With present knowledge it is impossible to prepare a satisfactory world classification, but this publication shows the diagnostic characters of what appear to be nameable groups.

4. Beetles. Keys to the genera of the Tribes Tenebrionini and Ulomini, which include the mealworms and flour beetles, have been published. This information should aid considerably in the identification of these difficult groups.

5. Cockroaches. An illustrated report on the cockroaches collected on an expedition by Brazilian scientists into northeastern Brazil has been published in Portuguese (jointly with a Brazilian specialist). A number of little known and potentially important species was encountered.

6. Grasshoppers. Research under P. L. 480 Project S9-ENT-6 (Uruguay) on the collection, identification and classification of grasshoppers has progressed satisfactorily. An illustrated manuscript describing a male allotype has been prepared for publication, the male genitalia of about 25 species have been illustrated, and several collecting trips have been made.

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Basic Studies to Name and Describe Beneficial and Injurious
Insects, Mites and Ticks

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FOREIGN EXPLORATION, INTRODUCTION, AND
EVALUATION OF BIOLOGICAL CONTROL AGENTS
Entomology Research Division, ARS

Problem. Many of the most serious insect and weed pests in the United States have been accidentally introduced from foreign countries without the insect enemies that kept them under control in their native homes. Some of the harmful insects so introduced have been effectively controlled by later introduction of their parasites and predators. Foreign exploration for beneficial biological control agents of insects and their subsequent introduction, colonization, and evaluation in this country is now a well established practice in the control of introduced insect pests. The use of imported insects to control introduced noxious weeds, although a more recent practice, has shown much promise. The biological approach to the control of insect and weed pests has great potential. Therefore, further foreign exploration is needed and additional research is necessary on the biology, ecology, nutritional requirements and the most effective manner of utilizing natural control agents, if they are to be used to maximum advantage. There is growing concern by the public over the insecticide and other residue problems in foods and by conservationists over the potential hazards of insect control chemicals to fish and wildlife. More effective use of natural control agents in meeting destructive insect and noxious weed problems could materially contribute to the ultimate objective of overcoming the pesticide residue and other hazard problems associated with the use of chemicals for the control of insects and weeds.

USDA PROGRAM

The Department has a continuing program on the use of beneficial insects. Basic and applied research is conducted on insect parasites and predators of insect pests and on insects that attack weeds, including foreign explorations for beneficial species and their introduction, liberation and evaluation in this country. A laboratory is maintained at Nanterre (near Paris), France, for studies on the parasites and predators of agricultural pests that have accidentally been introduced from Europe into the United States. At a station in Rome, Italy, studies are in progress on insects attacking a number of weeds, including puncture vine, Scotch broom, Dalmatian toadflax, Mediterranean sage, Russian knapweed, and halogeton. The field station at Rabat, Morocco, where studies on insects affecting halogeton had been conducted, was closed in March 1963, and the entomologist in charge transferred to the laboratory at Nanterre, France. Research on insects affecting aquatic weeds, especially alligator weed and water hyacinth, is being conducted at the National Agricultural Research Center in Castelar (near Buenos Aires), Argentina. Early in 1963 an entomologist spent about 6 weeks in Barbados collecting a predaceous beetle for release in Hawaii against the sugarcane mealybug. In the United States a receiving station and laboratory is maintained at Moorestown, N. J., where major emphasis is given to receiving, propagating and transshipping insect

parasites to proper liberation points. A laboratory for receiving, studying, and liberating insects affecting range weeds is located at Albany, Calif. Studies regarding entomophagous insects are also conducted at Riverside, Calif., and Beltsville, Md. The work at Albany and Riverside is conducted in cooperation with the University of California and the California Experiment Stations.

The Federal scientific effort devoted to research in this area totals 15.0 man-years. Of this total, 2.8 is devoted to search for and importation of foreign parasites and predators of insect pests; 2.1 to search for and importation of foreign insect enemies of weeds; 5.2 to basic biology, physiology, nutrition and evaluation; 3.9 to receipt, liberation and establishment of foreign insect enemies of insect pests and weeds; and 1.0 to program leadership.

Twelve grants for P. L. 480 funds have been executed for projects directly concerned with the study of insect parasites and predators. Nine of these projects involve exploration for beneficial species that might be shipped to this country for trial and release against agricultural pests here. These projects are as follows: Two projects - one in India (A7-ENT-5) and one in Pakistan (A17-ENT-8) - are for a study of parasites and predators of rice insects; two in the same two countries (A7-ENT-9, India, and A17-ENT-7, Pakistan) are for studies on parasites and predators of corn borers; one in India (A7-ENT-1) is for similar studies on sugarcane borers. Two projects - one in Pakistan (A17-ENT-5) and one in Poland (E21-ENT-2) - are for studies of the biological control of scales and aphids on fruit. Two projects, A7-ENT-2 in India and S9-ENT-1 in Uruguay are for surveys of crop pests and their parasites. These 9 projects call for 40 professional man-years annually. The other three projects directly concerned with studies of insect parasites and predators are A7-ENT-8, India (mass propagation), A7-ENT-17, India (rhinoceros beetle), and S3-ENT-7, Brazil (catalogue). These three projects call for 8 professional man-years annually.

Grants for two P. L. 480 projects have been executed for studies on the biological control of weeds. Project A17-ENT-9, Pakistan, is for a general survey of insects affecting noxious weeds, and Project A7-ENT-7, India, is concerned with studies of insects affecting witch weed. These two projects require 5 professional man-years annually.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Search for and Importation of Foreign Parasites and Predators of Insect Pests.

1. Parasites and Predators. A survey for natural enemies of the cereal leaf beetle, Oulema melanopa, in Italy, France and Germany indicated that several egg predators, 2 larval parasites and a larval disease are important factors in regulating outbreaks of the pest in central Europe. The same parasites that attack O. melanopa also attack O. lichensis, a closely related species more commonly found on wheat than on oats.

Thirteen species of parasites or predators of four different insect pests were collected in Europe; a coccinellid beetle predaceous on the sugarcane mealybug was collected in Barbados, B.W.I.; 11 species of parasites or predators from 5 insect pests were obtained through P. L. 480 projects in India, Pakistan and Spain; and a parasite of the brown soft scale was obtained from entomologists in Israel. All of this material was sent to Moorestown, N. J., for screening, testing, and transshipment to liberation points throughout the United States. In addition an introduced pea aphid parasite was sent to Colorado and Utah, and several parasites and predators of the black scale were sent to Iran directly from California.

B. Search for and Importation of Foreign Insect Enemies of Weeds.

1. Enemies of Weeds. Two insect enemies of weeds discovered in Europe appear quite promising and intensive tests are being made at Rome, Italy, regarding their host relationships. They are Apion fuscirostre, a seed weevil which attacks Scotch broom, and Phrydiuchus topiarius, a weevil which feeds in the stems and crown of Mediterranean sage.

C. Basic Biology, Physiology, Nutrition and Evaluation.

1. Alfalfa Weevil. A high proportion of the larvae of Bathyplectes curculionis, an imported parasite of alfalfa weevil larvae, are destroyed by phagocytes. In large alfalfa weevil larvae parasitized by only one Bathyplectes, phagocytes killed 54, 62, and 65% of the parasites in collections from New Jersey, southern California and northern California. When host larvae were parasitized by more than one Bathyplectes, comparable figures were only 23, 27, and 32%. Reasons for the apparent partial immunity and similar reactions in closely allied hosts are being studied at Moorestown, N. J.

2. European Pine Shoot Moth. Collections of European pine shoot moth larvae have been made in northern Germany at Lingen and in the Rhine Valley near Hartheim for the last 2 years. A tachinid fly, Lypha dubia, has been the dominant parasite at Lingen. It is heavily attacked by secondaries at Hartheim, but not at Lingen. There may be a correlation between secondary parasitism and the intensity of the shoot moth infestation which is increasing at Hartheim, but subsiding at Lingen. Lypha overwinters as a puparium. Studies are being conducted regarding the best way to hibernate the species under laboratory conditions.

3. Face Fly. Although about 34,000 face fly larvae were collected in the 9 Departments of France, no parasites were recovered from them or their puparia. A parasitic staphylinid beetle, Aleochoa tristis, was recovered in small numbers from puparia obtained from cow chips utilized in a faunal study. The beetle does not appear very promising as a biological control agent, but its biology and habits are being studied. It can survive under very adverse conditions.

4. Cereal Leaf Beetle. A solitary, internal ichneumonid parasite, probably Thersilocus moderator, was the most important parasite of the cereal leaf beetle, Oulema melanopa, encountered in survey work in central Europe. Parasitization ranged from 12 to 77% at 12 collection points. Tetrastichus sp., a gregarious chalcid, which parasitizes Oulema larvae, was also important, with 44% parasitization recorded at one locality in Italy. A larval disease was prevalent and, where practical, farmers irrigate infested fields in Italy to increase the humidity and the incidence of larval disease.
5. Coccinella septempunctata. Studies are being conducted regarding the biology and food habits of the lady beetle, C. septempunctata, which appears to be a major factor in the control of the pea aphid and potato aphid in France. Two generations of C. septempunctata were reared between December 15, 1962, and March 13, 1963, in spite of the fact that some authors consider the species univoltine. An average of 300 eggs was laid per female. Cannibalism is a limiting factor.
6. Predaceous Wasps. Studies in France regarding predaceous wasps were continued with special emphasis on species nesting in sandy soil. Nests were excavated and the prey, plus the associated wasp egg or larva, was removed and held in an artificial cell covered with a glass slide. Details regarding larval development, construction of cocoons, and food requirements were thus determined for 12 different species.
7. Lygus spp. Studies regarding the parasites affecting lygus bugs are being conducted at Riverside, Calif., and limited observations of a similar nature are being made at Moorestown, N. J., and Paris, France. In southern California an egg parasite, Anaphes (Anagrus) ovijentatus, commonly parasitizes 50% of the eggs. A tiny braconid, Euphoriana uniformis, was reared from Lygus elisus in late summer when this species predominated, and from Lygus hesperus in late summer when this species became more numerous. Euphorus pallipes was reared from Lygus lineolaris in Moorestown and an undetermined Euphorus from Lygus rugulipennis in France.
8. Agasicles n. sp. Observations in Argentina indicated clearly that this multivoltine flea beetle, which is a suppressant of alligator weed in that country, does not necessarily go into diapause during the winter months. Consequently there should be no difficulty in synchronizing Agasicles n. sp. with seasons in the northern hemisphere, if it is introduced into this country.
9. Puncture Vine Weevils. The seed and stem weevils, Microlarinus lareynii and M. lypriformis, appear to be more effective suppressants of puncture vine in Italy than in France. The reasons for this are not clear. The parasites attacking the two species apparently are entirely different in the two countries.
10. Halogeton. Life history studies and intensive screening tests on Heterographis fulvobasella, insect enemy of halogeton, were continued at

Rabat, Morocco. Moths were attracted to black light in good numbers through September 10 thus providing an ample supply of eggs and larvae for feeding tests. The young larvae failed to survive on any test plants except halogeton and Mexican fireweed, indicating very strong host specificity.

11. Purslane. This weed, Portulaca oleracea, originally came to the United States from Europe or Asia. It is now widely distributed in this country and Canada. It is a pest of many irrigated crop areas in California. Studies are being conducted to determine the effect of the leaf mining sawfly, Sofus pilicornis, on the pest. The insect appears to be more active in the warmer inland areas than in the cooler, coastal region where the weed is most troublesome.

12. Thistles. Native thistles are much more severely attacked by native thistle head insects than is the introduced bull thistle, Cirsium vulgare. Feeding habits and life histories of the more common native thistle head insects are being studied in California.

13. Miscellaneous Insects. A list of 16 range weeds against which biological control work might be recommended has been compiled after a review of literature and a study of weed lists from 12 western States.

D. Receipt, Liberation and Establishment of Foreign Insect Enemies of Insect Pests and Weeds.

1. Alfalfa Weevil. The alfalfa weevil has continued its rapid spread throughout the East. Additional colonies of Bathyplectes curculionis from California, totalling about 5,000 adults, were liberated to hasten dispersion. About 9,000 Tetrastichus incertus were also released in the East, and 3,000 in California. This species was propagated in the laboratory. It is already established in the East and should prove valuable in California also. Bathyplectes anura from France was obtained in sufficient numbers for liberation for the first time. Forty individuals were released in New Jersey and 112 in Pennsylvania. The adult weevil parasite, Microctonus aethiops, was recovered in very encouraging numbers in New Jersey. A colony of 102 adults was released in New Jersey and 175 in North Carolina. Two hundred adults of Dibrachoides druso, a parasite which has not yet been recovered in this country, were supplied to the State of New Jersey for laboratory propagation and experimentation.

2. European Pine Shoot Moth. Releases of European parasites were continued against this serious pest in pine plantations. The following were collected in Germany and transshipped to the Forest Service in Michigan: 415 Pristomerus vulnerator, 612 Temelucha interruptor, 361 Orgilus obscurator and 379 Lypha dubia. Orgilus is established in this country. It is not yet known whether the other species have taken hold in Ohio and Michigan.

3. Brown Soft Scale. This pest of citrus remained at rather low population levels in Texas during 1962 due to high mortality caused by severe frosts

the previous winter. In 1963 it was again plentiful. Three species of parasites were propagated at Moorestown, N. J., and 944 Encyrtus lecaniorum, 660 Coccophagus cowperi and 1625 Aphycus sp., probably flavus, were sent to Brownsville, Tex., for colonization and experimentation. The first two species originated in Israel, and the last in California. Arrangements were also made to send a culture of the parasite Metaphycus stanleyi to Brownsville from the Citrus Experiment Station in Riverside, Calif.

4. Pea Aphid. Two parasites of this pest were released. Aphidius smithi, an important parasite from India which has become well established in California, was collected in that State and approximately 10,000 were sent to Colorado and 8,000 to Utah. Aphidius ervi was reared at Moorestown, N. J., and 225 adults were sent to Idaho, 275 to Washington and 485 to Belleville, Ontario, Canada.

5. Sugarcane Mealybug. This insect is of considerable importance in Hawaii. A predaceous coccinellid, Hyperaspis trilineata, is an effective control factor in Barbados. During January and February successful collections were made in Barbados and about 4,000 adult beetles were sent to Hawaii. Laboratory tests indicated that H. trilineata will also complete its development on the gray sugarcane mealybug which is sometimes abundant in sugarcane fields in Florida.

6. Balsam Woolly Aphid. Several balsam woolly aphid predators were collected in Europe and transshipped to the Forest Service via Moorestown as follows: 1,835 Laricobius erichsonii to New Hampshire, 942 Pullus impexus to Oregon, 779 Aphidecta oblitterata to Oregon, and 800 A. oblitterata to North Carolina. Laricobius is already well established in the major areas of infestation; Pullus is established in Oregon. Aphidecta is not positively established, although it is known to have successfully completed one generation in North Carolina.

7. Miscellaneous Insects. A few small lots of parasites were shipped for testing and release. Fourteen Opius rhagoleticolus and 6 Phygadeuon wiesmanni from Switzerland and 120 Opius ferrugineus from New Jersey were sent to California for use against the cherry fruit fly. Twenty-eight specimens of a species of Aphidius from Europe were sent to Maine for use against the potato aphid. About 1,000 Cryptolaemus montrouzieri were sent to Iran from California for release against the citrus mealybug, and a consignment of black scales, expected to yield good colonies of Metaphycus helvolus, M. lounsburyi, M. stanleyi and Scutellesta cyanea, was sent to the same country from California.

8. Puncture Vine. The seed weevil, Microlarinus lareynii, has been successfully introduced into 6 western States for the control of puncture vine, Tribulus terrestris. In 1963 it was also successfully colonized in New Mexico, Texas and the Hawaiian Islands. On the island of Kauai it attacks a native Tribulus, T. cistoides as well as T. terrestris. T. cistoides is a perennial, and on this host the weevil apparently requires no diapause, for it has a year around food source.

9. P. L. 480 Projects. Investigations of parasites, predators and pathogens of sugarcane borers and corn borers are being conducted in India under P. L. 480 Projects A7-ENT-1 and A7-ENT-9. Two parasites of sugarcane borers were sent from India to the United States. Cocoons of Apanteles flavipes yielded 1,743 adults and puparia of Sturmia inferens yielded 156 adults. Both species were transshipped to the Grain and Forage Insects Research Branch laboratory at Canal Point, Fla., for study, propagation and possible release. Two parasites of Heliothis sp. were also sent to this country, but only one, an unidentified tachinid, was recovered as an adult. Fourteen were sent to Tifton, Ga., for testing against Heliothis zea.

Shipments of balsam woolly aphid predators were received from two Forest Service P. L. 480 Projects, A7-FS-7, India, and A17-FS-5, Pakistan. The following material from India was transshipped to the Southeastern Forest Experiment Station in Asheville, N. C.: 859 Leucopis sp., 487 Tetrableps sp., 130 Chrysopa sp. and 22 hemerobiids. Shipments from Pakistan were also sent to Asheville. They comprised 487 Tetrableps nr. pilipes and 322 Tetrableps sp. Gypsy moth parasites from Spain, P. L. 480 Project E25-FS-10, were also handled for the Forest Service. Large numbers of puparia of the tachinid fly Tricholyga segregata were received. After the adult flies emerged, they were held for mating and then placed in suitable shipping containers for release in Connecticut by the Plant Pest Control Division. Up to June 30, 886 flies had been released.

10. Fall Webworm. Several lots of fall webworm parasites collected in this country were shipped to Yugoslavia. The fall webworm is now a serious pest in central Europe and it is hoped that American parasites will help to control it there. Collections of the fall webworm were made at several points in the United States and parasites reared from them in Arkansas by Dr. Tadic of Yugoslavia. The parasites were then sent to Moorestown, N. J., for shipment to Yugoslavia.

PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

Search for and Importation of Foreign Parasites and Predators of Insect Pests

Adlung, K. G. 1962. Observations on the occurrence of Tetrastichus turionum Htg. (Hym.: Euloph.) a parasite of Rhyacionia buoliana (Schiff.) (Lep.: Tortric.) (In German). Zeitschrift fur angewandte Entomologie 50(4): 455-462.

Basic Biology, Physiology, Nutrition and Evaluation

McGuire, J. U. and Sailer, R. I. 1962. A method of estimating face fly populations on cattle. ARS 33-80, 7 pages, 2 tables.

Janvier, H. 1962. Studies on nest building hymenopterous parasites of aphids. IV. The genus Diodontus. (In French). Ann. Sci. Nat. Zoology 12 Serie, Tome IV, fasc. 3, 481-516.

Receipt, Liberation and Establishment of Foreign Insect Enemies of Insect Pests and Weeds

Puttler, Benjamin and Coles, L. W. 1962. Biology of Biolysia tristis (Hymenoptera, Ichneumonidae) and its role as a parasite of the clover leaf weevil (Hypera punctata). Jour. Economic Entomology 55(6): 831-833.

General

Adlung, K. G. 1962. The effect of a DDT spray against the white fir budworm on the predators of the balsam woolly aphids. (In German). Zeitschrift fur angewandte Entomologie 50(2): 239-241.

Adlung, K. G. 1962. Occurrence of Bruchophagus spp. as pests of alfalfa and red clover in south and southwest Germany. (In German). Gesunde Pflanzen 15(3): 46-49.

Adlung, K. G. 1963. Collection of bark living insects. (In German). Ges. Mainz 2(2): 73-75.

INSECT PATHOLOGY
Entomology Research Division, ARS

Problem. Basic investigations on viruses, fungi, bacteria, nematodes and protozoa are needed to fully exploit the use of such microorganisms as an approach to insect control. There is much interest in the use of these natural insect-control agents to overcome the growing concern over chemical residues following the application of insecticides to agricultural crops and livestock, and the increasing resistance of some insects to certain insecticides. The utilization of pathogens to produce diseases in insect populations, and so reduce them and the damage they cause, is an approach that has already shown great promise. Microorganisms that are pathogenic for insects are generally very efficient when used properly. They are specific for their insect hosts and harmless to men and other vertebrates. Basic research is needed for a thorough understanding of insect pathogens, including their growth and nutritional requirements, their resistance to environmental factors, and their mutability and mode of action, both in the laboratory and the field. Such knowledge must be obtained before these organisms can be used effectively in the control of insect pests.

USDA PROGRAM

The Department's Pioneering Research Laboratory on Insect Pathology at Beltsville, Md., has a continuing basic research program on the growth, nutritional requirements and mode of action of viruses, bacteria and nematodes affecting insects. Studies are in progress on mutability-induced changes in virulence of insect diseases, and resistance of insects to diseases, including studies of the effect of the environment on the pathogens. A comprehensive reprint library on insect pathology is being assembled. Collections of all sporeformers and viruses known to cause disease in insects are being obtained from world-wide contributors. A service involving the diagnosis of unhealthy insects is now available to all Division, State, and University laboratories.

The program includes collaborative studies with the Pesticide Chemicals Research Branch on instrumentation for monitoring insect activity, internal temperatures of insects, and effect of gaseous atmosphere on metabolism and development of insects. Collaborative studies are also under way with the Pioneering Research Laboratory on Insect Physiology on the effect of microorganisms on insect sterol requirements and on the structural differences of the eye in Vitamin A deficient house fly adults as determined by the electron microscope. Cooperative studies with the U. S. Naval Missile Center on effects of cosmic radiation on pathogenicity and enzymology of insect pathogens and changes induced in their nucleoprotein make-up are under way.

A cooperative project has been conducted with the Fruit and Vegetable Insects Research Branch, involving the electron microscopy of the two-spotted mite. A second study with this branch has been initiated to study the

serology of the non-inclusion virus of citrus red mite, in order to develop a tool for diagnosis of the disease.

The Federal scientific effort devoted to research in this area totals 9.0 professional man-years. Of this number 2.6 is devoted to virus diseases of insects; 4.5 to bacterial, protozoan, and fungus diseases of insects; 1.0 to nematodes and their associated bacteria pathogenic to insects and 0.9 to discovery and study of new pathogens.

At Poznan, Poland (E21-ENT-6) research under P.L. 480 funds amounts to 1.5 man years annually and at Madras, India (A7-ENT-20), to 3 man years annually.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Virus Diseases of Insects

1. Polyhedrosis of Pine Sawfly, Neodiprion pratti-pratti. Tests of purified polyhedra freeze-dried on lactose showed that infectivity of these polyhedra was much lower (about 1/100th) than that of unpurified polyhedra from the tissues of freeze-dried whole insects stored for a like period of time. The difference in infectivity does not seem to be due to attenuation of the virus since fresh polyhedral suspensions prepared from larvae killed by both groups of virus were equally infective. As earlier studies showed that a pathogenic bacterium is also associated with the virus infection in this insect, it is possible that the reduced infectivity might be due to the elimination of the bacterium by the methods of purification employed. Pure cultures of the associated bacterium isolated in 1955 during the original studies of the disease and held on oil-flooded slants were viable and satisfactory for further study of the phenomenon in 1962. The strong fluorescence under ultra-violet light shown by this organism furnishes a simple means of recognition. Material from this purified polyhedral preparation was furnished to the late Mr. E. E. McCoy, New Jersey State Department of Agriculture, for tests of the virus against Neodiprion pratti-paradoxicus. The negative results obtained might have been due to the low titre of the virus material rather than the lack of susceptibility of the species. A retest of this species should therefore be made.

2. Granulosis Virus Disease of Red-banded Leafroller. The red-banded leafroller (Argyrotaenia velutinana) is now under culture in the laboratory to permit assay of frozen stocks of the virus now on hand and for propagation of virus in amounts sufficient for resumption of laboratory and field studies of the disease. Tests in 1956 and 1957 showed that the disease could be successfully established in apple orchards under field conditions. The studies now in progress will explore dosage-infection relationships, temperature limits, host range, and changes in blood proteins of the host caused by the disease.

3. Bacteriophage of Pathogen of American Cockroach. In the examination

of diseased cockroaches (Periplaneta americana) submitted by the Insect Physiology Laboratory, several pathogenic bacteria were isolated. Cultures of one of the isolates showed plaque formation and from the plaques a highly potent bacteriophage was recovered. Various filters were tested for their ability to pass the bacterial virus and to retain the host organism, and by means of a Morton filter bacteria-free active filtrates were obtained. Cultures of both susceptible and non-susceptible strains of the bacterium were also isolated. Injection tests demonstrated that both were pathogenic for wax moth larvae and that injection of the bacteriophage significantly reduced the effect of a challenging dose of the susceptible strain. After these preliminary studies were completed, cultures of the bacteria were made on oil-flooded slants and tubes of the bacteriophage were sealed and frozen to preserve the material for future study.

4. Abnormal Growth in Virus-infected Tissue. The previous annual report suggested the possibility of abnormal mitoses in fat cells of seventh instar larvae of the salt-marsh caterpillar infected with granulosis virus. It has now been established that no mitosis occurs in uninfected larvae in the last instar. However, excessive mitosis occurs in virus-diseased larvae, in fat cells adjacent to granulosis-infected cells.

It was postulated that a substance may be produced by infected cells that encourages mitosis in uninfected cells. Accordingly, water and chloroform ether (1:1) extracts were prepared from fat bodies of granulosis-infected, last instar larvae, and the water extracts were freeze-dried and concentrated in sterile water before injection into groups of uninfected last instar larvae. After two days, fat bodies of the injected insects were removed and prepared histologically for examination. There was no evidence of excessive mitosis. Consequently, no water-soluble material is produced by granulosis-infected fat cells that causes mitosis. The examination of the fatty materials is currently under way.

5. Virus Stock Culture Collection. Collection of insect viruses has continued. The laboratory now holds some 33 species of virus (several in substantial quantity, including the cabbage looper polyhedrosis, the red-banded leafroller granulosis, the salt-marsh caterpillar granulosis, and the Virginia pine sawfly polyhedrosis). Considerable effort is being made to obtain all known cytoplasmic polyhedroses since these viruses have a record of ability to cross-infect between related species of hosts. Attempts to infect the European corn borer with these microorganisms are under way.

B. Bacterial Pathogens of Insects.

1. Taxonomic Studies of Crystal-forming Bacteria. Twenty-eight separate isolates of crystal-forming bacteria including strains from Russia, Germany, Japan, France, Spain, Switzerland, Scotland, Kenya, Canada and the United States have been submitted to some thirty distinct biochemical, serological, and electrophoretic tests. Several discrepancies in the identification of

these bacteria have been detected and differences in toxin production are now known. A forty-eight-hour cell-esterase identification system has been established that should be of immense value as a diagnostic and epizootological tool. A paper of these findings is now in preparation.

2. Mode of Action of Crystal-forming and Non-crystal-forming Bacteria of Bacillus cereus and B. thuringiensis Group. (a) Bacillus thuringiensis var. thuringiensis and certain strains of this organism produce an exotoxin, the so-called fly factor, when grown on adequate media. This toxin can now be separated and purified by means developed at this laboratory in cooperation with the Insect Physiology Laboratory. The toxic component is strongly U.V. absorbent and exhibits a characteristic triple peak in the vicinity of 270 mu. These peaks are identical with Ca-dipicolinate suggesting that the non-toxic, pyridine salt is at least a marker for the toxin. Since the amount of 270 is proportionate to the biological activity in killing the house fly, an in vitro spectrophotometric test to determine the level of toxin in commercial preparations should be possible.

The fly factor interferes with pupation in the house fly Musca domestica, the face fly M. autumnalis, and the mosquito Aedes aegypti. The toxin is produced in cultures during sporulation or in the presence of dipicolinic acid, suggesting that a precursor of the toxin is formed before sporulation. This precursor may be harmful to young house fly larvae and the mode of action may be different from that which affects the prepupa of the house fly. Twenty-eight varieties of B. thuringiensis, B. entomocidus and B. finitimus were tested for exotoxin production. Only strains of B. thuringiensis (fourteen) produced the toxin. The remainder proved harmless to the house fly in tests involving some 12,000 larvae. According to other workers, this toxin can be given as a feed additive to cows and chickens and appear unchanged in the feces, preventing complete development of house flies in manure. This material, in this way, may be of considerable interest commercially. A paper on these findings is in the final stages of preparation.

In cooperation with the Apiculture Research Branch, preliminary tests were carried out with adult honey bees fed the fly factor toxin from Bacillus thuringiensis. At very high concentrations (200 mg. of crude exotoxin : 10 ml. of sucrose solution) the exotoxin proved fatal to the bees in 72 hours. This concentration is higher than could ever occur naturally in fields treated with B. thuringiensis.

(b) Cell-cementing substances in the midgut of insects susceptible to crystal-forming pathogens have been tentatively implicated, on histological grounds, as the pathogenesis of these disease agents. The mucoid cell-cementing substance of the midgut of Galleria mellonella has been isolated to further examine this hypothesis. The isolation procedure involved the removal of gut lipids in 4:1 ethanol chloroform followed by the solubilization of the remaining extraneous tissue by papain and a final trypsin, chymotrypsin proteolysis. A selective "salting out" procedure was used to separate the mucopolysaccharides that remained. This routine yielded a

fraction that corresponds to the polysaccharide, hyaluronic acid. Hyaluronic acid is, incidentally, one of the major cell-cementing substances obtained from vertebrates. Only traces of mucopolysaccharides were precipitated out of the insect material at salt concentrations used by other workers to obtain heparin and chondroitin sulfates. Preliminary analyses of the first fraction have established the presence of both uronic acids and amino sugars. The small amounts of the latter fraction have, to date, precluded their complete identification. Further extractions are in progress, and means to detect the break-down components of the hyaluronic-acid-like component in the infected insect gut are under investigation.

(c) Preparation of "clean" crystal preparations from crystalliferous bacteria are essential since investigators are now generally convinced that various strains of bacteria produce crystals of varying activity for different insect species. Several hundred milligrams of crystals have been produced during the year. Considerable amounts of these materials have been sent to laboratories in this and other countries to carry out cooperative studies.

Tests on the silkworm and the salt-marsh caterpillar with crystal preparations show that commercially produced materials are lower than expected in crystal toxicity. Crystal counts of these same preparations are approximately 1 crystal to two spores, which would not explain the lack of activity compared to laboratory produced material. This phenomenon is under investigation.

3. Clostridial Pathogens of Insects. (a) Extensive investigation of Clostridium malacosomae have been conducted, using most of the commercially available anaerobic media, in an attempt to propagate the bacterium. None of these experiments has led to a better medium than Bucher's. This completes the first phase of this investigation. An examination of the tent caterpillar gut contents is being conducted to detect and isolate the growth factor that permits growth of this fastidious organism.

(b) Since Bucher isolated three species of Clostridium from infected larvae of the tent caterpillar (Malacosoma pluviale), interest in the clostridia associated with insects has been stimulated. Special methods are required for isolating these fastidious pathogens from insects. Infected specimens are frequently grossly contaminated with aerobic and facultative bacilli. A device for the selective culture of clostridia has been developed. It is a simple compartmented culture tube consisting of an inner tube of borosilicate glass 7 mm. outside diameter and 115 mm. long. The inner tube is flared slightly at the top, flared and indented at the bottom, and perforated with four small holes about 25 mm. from the bottom. When the inner tube or separator is placed in a conventional culture tube containing liquid medium, two compartments are formed, one inside the separator and one between the separator and the culture tube. Infected insect material is delivered to the culture tube (outer compartment) with a long tipped Pasteur pipette. Isolations are made from the inner compartment to

fresh media. By this method, the isolation pipette does not contact the surface-growing aerobes in the outer compartment and permits enhanced and selective growth of clostridia from grossly contaminated material.

4. Bacterial Phagocytosis by Insect Blood Cells. The most prominent protective mechanism in insects is the phagocytosis of foreign bodies by certain types of blood cells. Some recent evidence indicates that the phagocytic activity of these cells may be increased following an intra-hemocoel injection of certain kinds of foreign materials. This suggests the formation of opsonin-like materials similar in function to those produced in mammals. Opsonins are antibodies which render microorganisms more liable to phagocytosis.

In vivo and in vitro tests are currently being conducted to compare the rate of phagocytosis of bacteria coated with immunized serum with bacteria coated with normal serum. Immune serum is prepared by injecting a group of insects with bacteria for a period deemed necessary for the production of the material in question. The blood is then extracted and centrifuged to remove the cells. If the presence of opsonins is established, electrophoretic separation and characterization may be attempted.

5. Effect of High Altitude Radiation on Spores of Insect Pathogens. The studies in cooperation with the Life Sciences Department, U. S. Naval Missile Center, were continued, and a broader protocol of study to include collaborative investigation of changes in the nucleoprotein make-up of exposed spores and their progeny was developed. A fourth payload (S/N A-6) of the BUWEPS HUGO III Program was successfully launched at Point Mugu, Pacific Missile Range on July 10, 1962, but again the payload was not recovered. No further launches were attempted under this program, and the program was terminated in September 1962. While no recovery was made under this program and the objective of the tests could therefore not be realized, much experience was gained that should be valuable in future studies.

A protocol based on the experience with the HUGO III Program was submitted in November 1962 as a biological experiment in space for the biological satellite program of the National Aeronautics and Space Administration. The experiment received favorable consideration but was deemed more suitable for high altitude balloon studies since these cheaper vehicles would provide adequate exposure and problems incident to recovery are much simpler. To obtain preliminary observations and to check on the methodology of the experiment, biopacks similar to those designed for the HUGO III Program were piggybacked in payloads of two high altitude balloon flights conducted by Carl E. Fichtel, Goddard Space Flight Center, NASA. The balloons, NASA Flight numbers 1040-N and 1042-N, were launched from Brownwood, Tex., on January 21 and 28, 1963. The first, aborted after 3 hours at altitude, and was recovered the following day at Roswell, N. Mex. Flight 1042-N completed the scheduled 6-hour flight at altitude and the payload was recovered minutes after touchdown at Odessa, Tex.

These tests, including laboratory and transportation ground controls, consisted of 24 biopacks. Of these, 6 biopacks were flown in each of the two flights, 3 containing spores of Bacillus popilliae and 3 containing spores of three strains of B. thuringiensis. All 24 biopacks contained eggs of Aedes aegypti in addition to spores of one of the bacterial pathogens. A few days after the return of the biopacks, unfortunate excessive temperature that occurred in the laboratory invalidated the test in respect to the mosquito eggs. Fortunately, the excess temperature did not affect seriously the nuclear emulsion monitoring plates and would have no effect on the spores. Hence valid results should be forthcoming in respect to the primary objective of these tests.

6. Rickettsiella popilliae. Studies with adult Japanese beetles show that the course of this disease in the adult is similar to that in larvae. The rickettsia attack only certain cells. These cells develop the typical blue coloration observed by dark field examination and contain numerous crystal-line inclusions. Because of the opacity of the adult cuticle, the gross symptoms apparent in the larvae are not evident in the adult, but the microscopic picture is similar. At high dosages, examination of the blood removed by thoracic puncture shows large numbers of typically infected cells about 14 days after injection. The infected beetles are quite active at this time and are capable of normal flight. Diseased beetles are long lived and continue to feed until a few days before death. Females containing well developed eggs were dissected, and in spite of the enormous numbers of rickettsia present in the hemolymph, none were found inside the eggs. the beetles in these tests were not provided with soil, no eggs were deposited. An adequate number of tests were made with Japanese beetle adults to demonstrate that this stage could be used as an assay for viable infective rickettsia. The number of beetles available from field collection and their longevity decrease rapidly after peak emergence. Third-instar larvae reared from eggs in formaldehyde-treated soil were therefore used for this purpose.

Frozen tissue cultures, representing first and third passages of Rickettsia popilliae through human synovial cells, supplied by Earl Suitor, Virology Division, U.S. Naval Medical Research Institute, were thawed and broken up with and without sonication and injected at several dilutions into disease-free Popillia larvae. Portions of the frozen rickettsial suspensions used as the inocula of the tissue cultures were injected at several decimal dilutions so that dosages from 6 rickettsia to 6 million rickettsia per larva were obtained for comparison. A portion of this suspension was first sonicated to determine the effect of this treatment on the rickettsia. To conserve material, dosages employed with the sonicated material was from 6 to 600,000 rickettsia per larva. Sonication was performed at 1/10 dilution of the stock suspension, since 30 ml. of suspension was required. The results of these tests indicated but slight damage to the rickettsia due to sonication since 11 of 25 larvae injected with 6 sonicated rickettsia developed infection as compared to 25 of 25 injected with unsonicated material at the same level, whereas consistent infection was obtained with both suspensions at higher dosages. While positive infection was obtained with both tissue culture preparations at low dilutions (1/10 with culture No. 37-B, 1/100

with culture No. 43-1), the estimated numbers of viable rickettsia present in the cultures were very low and much less than those expected to remain by straight dilution of the large number of rickettsia (100,000,000) used as the original inoculum of the cultures. While it is possible that the rickettsia might have made some growth in the synovial cells, the amount of this growth would have been very slight, and would not constitute propagation of the agent. It is possible that larger numbers of rickettsia avirulent for the insect host might have been present in the cultures, but there is little microscopic evidence to support this. Typically infected larvae from all series have been frozen so that suspensions of rickettsia might be prepared and compared for infectivity by reinoculation at equivalent dosages.

C. Nematodes and Their Associated Bacteria Pathogenic to Insects.

1. DD-136 Nematode for Control of Codling Moth. The field test was continued in the Kearneysville, W. Va., orchard, in cooperation with entomologists of the Fruit and Vegetable Insects Research Branch, to determine whether applications of the DD-136 nematode would provide effective control of the codling moth. As in previous years, a very high degree of control was obtained in larvae under bands. Bark scraping of the trunks and scaffold limbs above the bands indicated that nearly equal control of larvae was obtained in these areas also. Nevertheless, a higher degree of fruit damage was experienced in young apples and at harvest than would be indicated from the degree of larval control attained. Fruit yields in the orchard in the fall of 1962 were high and in spite of the large number of apples on the trees at harvest, more than 40% of the picked fruit were wormy. The large population of larvae cocooning on the trunks showed over 75% nematode kill under bands by mid-October, and bark scrapings far up the scaffold limbs of sample trees showed a similar rate of kill under bark scales. The larval population was further reduced by early spring. A trunk survey in late April 1963 showed many old dead nematode-killed larvae, very few recently killed larvae, and a relatively small number of apparently healthy larvae. Two weeks following a nematode application in May 1963, nearly all overwintering survivors were found dead of nematode infection. Only a negligible number of the overwintering larvae in the orchard could have emerged as moths following this treatment. Nevertheless, an appreciable first brood infestation was present in the comparatively small number of fruits set. The large unsprayed orchard directly across the road from the test orchard and less than 250 yards removed from it had a fairly heavy load of apples last fall and undoubtedly contributed ovipositing moths to the test orchard this spring.

D. General.

1. Monitoring Electrophysiological Signals and Locomotor Activity of Insects. In cooperation with the Pesticide Chemicals Research Branch, the study of the electrophysiological and locomotor activity of the Madeira cockroach, Leucophaea maderae, to detect biological rhythms, has been con-

tinued and has now been submitted as a formal proposal for a biological experiment in space to the National Aeronautics and Space Administration for inclusion in its biological satellite program. The experiment is now under active consideration and counterpart experiments are being conducted at NASA's Ames Research Center at Moffitt Field, Calif.

A simple experimental infra-red detector system for recording and evaluating locomotor activity of house flies was constructed and demonstrated to personnel of the Orlando Laboratory, Insects Affecting Man and Animals Research Branch, for use in estimating the relative locomotor activity of chemically sterilized males versus normal males. The monitoring beam of infra-red was obtained by means of a Corning heat transmitting filter, and the test chamber is also provided with visible light that can be regulated to control the general level of activity.

2. Histopathology of House Flies fed Carcinogenic and Noncarcinogenic Analogues of 2-fluorenamine. In cooperation with the Insect Physiology Laboratory, histopathological examination of house fly larvae and pupae fed carcinogenic 2-fluorenamine derivatives revealed benign tumors similar to those hereditary tumors reported by other workers. Noncarcinogenic derivatives did not cause tumor formation. This suggests the possibility of using the house fly as a preliminary screening animal for carcinogenic materials. The large number of generations and sensitivity of these species would seem suitable for large scale testing operations. The tumors are usually found in the hemocoel and they consist of large rounded or polygonal cells scattered throughout a fibrous-like stroma. In older tumors the more centrally located cells become necrotic and are replaced by melanin deposits.

3. Histopathological Changes in Apholate-treated Drosophila. Histopathological examinations were made of gonadal tissues of Drosophila melanogaster adults previously treated with apholate and gamma radiation. In the treated testes there was a cessation of sperm production at the anterior end with a general necrosis of the germinal epithelium that progressed, in time, over the entire tubules. High level of either treatment halted production of viable eggs. Microscopic examination of Feuglen-treated ovarioles revealed very small Feuglen-positive clumps of chromatin, which indicated that complete breakdown of the nurse cells, oocytes, and follicle cells had occurred.

4. Electron Microscopic Investigation of the Structure of Rhabdoms of the House Fly Eye. Flies fed on a diet depleted of Vitamin A could perceive motion but not color. Biochemical and morphological studies by other investigators have indicated that Vitamin A is probably a component of the rhabdom wall structure. It was assumed that differences in this structure between control and Vitamin A free diet reared flies would be on the biochemical level or perhaps, if gross enough, might be detectable by electron microscopic examination.

Flies were reared aseptically on the following diets: (1) standard CSMA media, (2) CSMA media minus Vitamin A, and (3) CSMA media minus B-carotene.

Adult females were selected and amputations of eyes were performed in cold 2% osmium tetroxide fixative. Tissues were fixed 1-2 hours at 4°C., rinsed, dehydrated through an ethanol series and embedded in the following media: maraglas mixture C according to the method of Freeman and Spurdock; Epon according to Luft's method; and a methacrylate mixture (1 part methyl : 3 parts butyl). Difficulties were encountered in infiltration with each of the embedding materials used. Sections made from maraglas embedded tissues were stained with a saturated alcoholic solution of uranyl acetate to enhance the contrast. Methacrylate embedded tissues were stained with a 1% aqueous solution of uranyl acetate. Preliminary examinations have indicated that no gross morphological differences exist between rhabdoms from flies fed the control and Vitamin A free diet.

5. Investigation of Cuticle of Two-spotted Spider Mite. A cooperative project was initiated with the Fruit and Vegetable Insects Research Branch for the electron microscopic examination of cuticle from mites - nonresistant and resistant to organophosphates. Initially, whole female adult mites were fixed in osmium tetroxide, dehydrated and embedded according to standard techniques. The tissues observed were poorly fixed and inadequately infiltrated with the embedding medium. Next, mites were dissected on dry ice in the region of the cephalothorax, fixed in osmium tetroxide, dehydrated in an ethanol series, and embedded in a methacrylate mixture (90 parts butyl : 10 parts methyl).

The nonresistant mite integument is composed of an outer epicuticle, an exocuticle, a laminated endocuticle, a hypodermis, and a basement membrane and is morphologically similar to the insect integument. In many of the sections observed the cuticle was modified into cuticular extensions with lobed apices. These appear to be noninnervated and are undoubtedly similar to the immovable non-cellular processes already described on insects by other workers. Measurements of a few electron micrographs indicated the possibility of a difference in thickness in some of the cuticular components between resistant and nonresistant mites.

PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

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BEES AND OTHER POLLINATING INSECTS

Entomology Research Division, ARS

Problem. The pollination of some 50 seed and fruit crops depends on an abundance of honey bees and other pollinating insects. Although certain wild bees help to pollinate cultivated crops, honey bees are estimated to account for three-fourths of the pollination by insects. Most growers are not fully aware of the importance of honey bees and the wild bees in the production of pollinated insect crops. Therefore, research that leads to more efficient and economical production of honey bees is imperative to insure effective pollination of many crops and the economical production of honey. A problem of major significance is the increasing use of pesticides, many of which are hazardous to bees or destroy important pollen and nectar sources. There is need for more knowledge of the management of bee colonies; breeding of improved hybrid bees; physiology and behavior of queens, drones, and workers; and the various diseases and pests of the honey bee and means for their control. There is also need to study the many facets of the complex pollination problem to integrate effectively populations of honey bees and other pollinating insects with crop needs and practices. More knowledge should be obtained about wild insect pollinators and their management. It is also essential to study the effects of farm practices, such as the use of different pesticides, changes in crops, soil management, and harvesting, on the economy of the beekeeping industry and the survival of pollinating insects, and to develop procedures to minimize losses from such practices. Information is needed on nectar and pollen plants for use in conservation program efforts to provide bee forage areas in wastelands, watersheds, and roadsides. The nutrition of bees and the nutritive value of different pollens to bees require intensive investigation together with basic nutrition studies for development of pollen substitutes.

USDA PROGRAM

The Department has a continuing program involving apiculturists, geneticists, microbiologists, physiologists, and entomologists, engaged in basic studies and in research concerned with the application of known principles to the solution of crop pollination problems for the farmer and problems that affect the beekeeper. Bee breeding investigations at Baton Rouge, La., are cooperative with the State Experiment Stations of Louisiana, California, and Wisconsin, the Honey Bee Improvement Cooperative Association, and the Ontario Agricultural College, Guelph, Ontario, Canada. Bee management investigations at Madison, Wis., are cooperative with the Wisconsin and Arizona Experiment Stations, the Honey Bee Improvement Cooperative Association, the Department of Apiculture at Ontario Agricultural College, Canada, the Abbott and Pfizer Laboratories, the Eastern Utilization Research and Development Division, and the Agricultural Engineering Research Division. Investigations on bee diseases are carried on at Beltsville, Md., and Laramie, Wyo., in cooperation with the Louisiana, Wisconsin, and Wyoming Experiment Stations. Honey bee pollination investigations at Tucson, Ariz.,

are carried on in cooperation with the Experiment Stations of Arizona, California, Louisiana, Utah and Wisconsin. Wild bee pollination investigations at Logan, Utah, are conducted in cooperation with the Experiment Stations of Arizona, Utah, Louisiana, Wyoming, Idaho, Oregon, Washington, the Crops Research and Agricultural Engineering Research Divisions, ARS, and private beekeepers and farmers.

The Federal scientific effort devoted to apiculture research totals 22.1 professional man-years. Of this number 6.7 is devoted to breeding and management to improve productivity in honey bees; 4.7 to biology and control of diseases and pests of honey bees; 5.5 to behavior and utilization of honey bees and other insects in crop pollination; 2.7 to effect of pesticides and other farm practices on honey bees and other pollinating insects; and 2.0 to program leadership.

P. L. 480 grants total 9.25 man-years. Bee breeding research is being conducted under P. L. 480 funds at the Central Apicultural College, Warsaw, Poland (2.5 man-years) and at the Faculdade de Filosofia, Ciencias e Letras de Rio Claro, Rio Claro, Sao Paulo, Brazil (3 man-years). Bee disease research under P. L. 480 funds is underway with the Government Agriculture College and Research Institute, Ludhiana, Punjab, India (3 man-years) and with the Instituto Nazionale di Apicoltura, Bologna, Italy (3/4 man-year).

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Breeding and Management of Honey Bees

1. Genetic Studies. Crosses between gentle and vicious lines of bees were tested in colonies and nuclei. Studies at Baton Rouge, La., indicated that temper in bees is probably determined by several genes with additive effects.

At Baton Rouge, three chemosterilants, tepa, metepa and apholate were fed to fertilized laying queens in mailing cages with attendant bees in concentrations of 0.1, 0.5, and 1.0% in a sugar solution for 1 or 2 days. All queens soon died except three fed metepa at 0.1, 0.1, and 0.5%, respectively. Oviposition was delayed when these queens were first reintroduced to their hives and hatchability of eggs was greatly or completely reduced. In all cases oviposition and viability to emergence was back to normal by the 48th day. Three colonies were fed 400 cc of 0.5% solutions of the three chemicals. The queens in colonies fed tepa and apholate laid sterile eggs to the ninth and tenth days, respectively. Then hatchability of the eggs and viability of the brood gradually increased until recovery was complete after 48 days. Metepa reduced viability of the brood only to 60% and recovery was also complete in 48 days.

Various stocks of bees tested for resistance to DDT at Baton Rouge differed only slightly in LD 50. A resistant stock from the Citrus Experiment Station at Riverside, Calif., was less resistant than some of the others

tested and two generations of selection have not materially increased resistance in this stock. Methods for testing individual worker bees as well as queens and drones have been developed, which give more repeatable results than the former method of testing many bees in a cage.

At Baton Rouge, eight lines of honey bees were tested for variances in egg weight and numbers of eggs laid by queens. Highly significant differences between lines were found in both variables. The environmental factors -- colonies, groups and weeks -- contributed significantly to the number of eggs laid by individual queens. There was no significant correlation between the number of eggs laid by individual queens. There was also no significant correlation between the number of eggs laid and the weight of these eggs.

Bee breeding research is being conducted under P. L. 480 funds at the Central Apicultural College, Warsaw, Poland (E21-ENT-7). This work has shown that in an inbred colony with brood having about 50% viability all the eggs hatched and then about 50% of the larvae disappeared from the cells within 24 hours. Histological investigations showed that the larvae which disappeared were diploid drones produced from fertilized eggs. Thus, it was shown for the first time that diploid drones can develop from fertilized eggs. It was further shown that diploid drone larvae were eaten by the bees while the larvae were still alive. Some of these larvae were reared in an incubator without the attendants and more than one half of them were still alive after 2, 3 or more days, and more than one half of them were alive after 5 days. Research on bee breeding is also being conducted at the Faculdade de Filosofia, Ciencias e Letras de Rio Claro, Rio Claro, Sao Paulo, Brazil (S3-ENT-1). Significant findings on this project have been: An average of 1.38 lethal equivalents per bee was found in Apis mellifera mellifera and total mutation rate for deleterious genes is 7.5%; the dying larvae of 50% viable brood A. mellifera are females; queens of stingless bees are regularly fed with (in order of importance) worker eggs, glandular food put in the alveolus, and food given by workers.

2. Hybrid Bees. At Madison, Wis., the (ZX)(M1) hybrid produced 40 pounds more honey than other hybrids in the same test. The respective average yields obtained from the six hybrids (ZX)(M1), (DyZ)(M1), (6Z)(M1), (6X)(M1), Starline, and Midnite, were 285, 201, 246, 212, 218, and 148 pounds of honey in excess of their 12-month consumption. The (ZX)(M1) and (DyZ)(M1) hybrids ranked superior to all others on the basis of temperament, behavior, swarming tendency, use of bur comb, fall weight of brood nests, and brood area measurements. The (ZX)(M1) hybrid is a superior stock and will be released to the industry as soon as the mechanics of release are worked out. The hybrid breeding research has been in cooperation with the Department of Apiculture, Ontario Agricultural College, and the Honey Bee Improvement Cooperative Association. A conference was held July 8-9 with Dadant & Sons, Hamilton, Ill., to discuss the possibility of a cooperative agreement to facilitate release of Government stock. A proposal was drawn up for consideration in the Department of Agriculture.

At Madison, Wis., ten inbred lines have been maintained, breeder queens produced, and many hybrid combinations of these inbreds made for prescreening tests to determine their combinability and colony characteristics. Outcrossed inbred queens are difficult for commercial queen breeders to use. Use of 3-way hybrid breed queens (6Z)(X) to establish vigor in the breeders (grafting mothers) appears to be a sound practice. Lines 6 to Z are of similar stock origin, and (6ZX)(M1) stock performed as well as (ZX)(M1) in prescreening tests. Backcrossing (ZX)(ZX) to obtain greater vigor in the breeding queens is also being studied.

3. Overwintering Queens. Substantial progress has been made at Madison, Wis., with the overwintering of caged queens in multiple queen storage colonies, but certain problems have not been completely resolved. Queens, once accepted by the bees, depend for their survival upon a large population of bees, protected from Nosema infection, with the caged queens and food supply organized so that the winter cluster cannot pull away from the queens under severe, low temperatures. The population must be composed of relatively young bees in late fall so that they can survive in suitable numbers until early spring.

4. Smoking Bees. At Baton Rouge, La., the effect of a social disturbance of the honey bee colony was measured during the honey flow period for the seasons 1961 and 1962. The weight gains of disturbed colonies were compared with those left undisturbed. Colonies disturbed gained weight on the day of treatment but less than colonies not disturbed. The differences in weight gains were highly significant.

At Madison, Wis., the use of smoke from ammonium nitrate fertilizer placed on the hot coals of a smoker is a practice of some beekeepers in management of colonies for honey removal, colony manipulations, and requeening operations. Adverse effects on colony populations often result but are overlooked. In both cage and field tests, longevity of the bees was reduced about 50 percent by this practice. Toxicity is apparently due to nitric acid and ammonia formed in the decomposition of ammonium nitrate.

5. Removing Bees from Supers. At Tucson, Ariz., propionic acid and anhydride mixtures were superior to water dilutions for removing bees from supers. Experiments at Madison, Wis., show that dilution of propionic anhydride with water to obtain dispersion is not necessary. Propionic anhydride was applied full strength to the fume chamber pad and the use of one fume chamber allowed the removal of 25 shallow supers in 30 minutes on a clear, sunny day at 80° F.

At Madison, Wis., a method of removing supers with dilute glacial acetic acid and smoke was more effective than propionic anhydride at temperatures below 60° F. A high-volume, low-pressure air blast was also effective in removal of bees from honey supers. Prof. Gordon Barrington, University of Wisconsin, Agricultural Engineering Department, proposed and provided equipment consisting of a portable motor generator and an industrial vacuum

blower unit. Over 500 supers were removed with this equipment. One-half to one minute was required for each super. The supers had consistently fewer bees left in them than by any other method, including those using repellents.

At Tucson, Ariz., methods of screening attractants and repellents have been improved. In tests with 84 compounds 10 showed some degree of repellency. Two materials were more repellent than propionic anhydride. Seven materials showed more attractancy, two of which were outstanding in this respect.

6. Watering Bees. At Madison, Wis., a sanitary water supply for bees was provided by 30 square feet of synthetic sheet sponge floated in a tank. No bees can gain access into the tank and sanitation can be maintained by sterilizing the sponge. A wheelbarrow equipped with a water vat and rack for supporting hive bodies proved very efficient for cleaning up bur comb without stimulating robbing. (Bees able to contact exposed honey in times of scarcity of nectar will ordinarily become very excited and stimulated to robbing with accompanying stinging).

7. Wax Melting. At Madison, portable infrared gas heaters were adapted for melting beeswax. With this method scrap wax can be rendered inside the building under all weather conditions while the solar extractor requires sunlight.

8. Honey Quality and Refrigeration. Small samples of each lot of honey extracted at Madison were graded, and duplicates placed at room temperature, under refrigeration, and in deep freeze storage for observation of change. After 6 months of storage, samples at room temperature showed noticeable darkening and flavor change, while those under refrigeration and in the freezer did not. Samples under refrigeration all showed granulation starting, while those at room temperature or in the deep freeze had no granulation.

B. Diseases and Pests of Honey Bees

1. Diagnostic Service. At Beltsville, Md., 1,358 samples were diagnosed for diseases during 1962, of which 944 were brood and 414 adult bee diseases.

2. Tropilaelaps clareae. This mite is not known to occur in the United States. At Beltsville, brood comb samples received from the Philippine Islands and Hong Kong contained the mite, later identified as T. clareae. The mites were found in sealed brood cells containing honey bee pupae that were dead on arrival. The pupae had defective or deformed wings and under the pupae on the base of the cell were a number of adult mites, sometimes as many as 6 or 8. The mites were also dead on arrival. No mites have been located on or in the dead pupae. Whether the honey bee pupa is directly attacked by the mites is unknown. This mite is reported to be an increasingly serious problem to the beekeepers in the Philippines and Hong Kong.

Following suggestions of the Apiculture Research Branch, Ismael P. Sevilla of Malolos, Bulacan, Philippine Islands, has used Folbex as a possible control for this mite. His fumigation tries with methyl bromide, Tedion V-18, tobacco smoke and methyl salicylate were unsuccessful, and caging the queen to provide a broodless period did not control the mites. On the other hand, according to C. Y. Yip of Hong Kong, caging the queen for more than ten days during which period no brood is reared has successfully cleaned the combs of mites.

3. External Acarine Mites. At Beltsville, Md., the external mite, Acarapis dorsalis, was found on bee samples from Saskatchewan, Canada. Package bees shipped from Louisiana to Wyoming were infested with eggs and with immature and adult forms of A. dorsalis and A. externus mites.

4. Acarine Disease. No Acarapis woodi mites that cause the Acarine disease of honey bees have been found in the United States.

A survey made under P. L. 480 project A7-ENT-10 in India showed no A. woodi mites on about 100 colonies of Apis dorsata (giant honey bees) or A. florea (dwarf honey bees). A. woodi mites were found in 4 provinces on Apis indica (Indian honey bee). The occurrence of this mite in India is more widespread than was expected.

In the P. L. 480 project in Italy (E15-ENT-1) 3 of 15 materials tested proved to be nontoxic to bees and showed acaracidal effect of promise. They were: Chlorparacide, camphor + 5% safrol oil + 5% nitrobenzene, and champhor + 10% mustard oil. Examination of individual bees demonstrated that, when the infestation is very serious, the mites will spread extensively throughout the respiratory system of the thorax and the head (where they are more protected from the fumigant substances which constitute the traditional methods of control), instead of stopping in the prothoracic tracheae trunks, their habitual resting place. They may also transfer from the tracheae on one side of the thorax to those on the other side. The blood of healthy and infected bees revealed some differences both as to quantity and structure, which will be further investigated.

5. Gregarines. Gregarine parasites from the ventriculus of honey bees at Baton Rouge, La., appear very similar to specimens found several years ago at Laramie, Wyo., and St. Paul, Minn.

6. Virus Paralysis. At Laramie, Wyo., bee samples received from a colony showing paralysis in Washington State were crushed and filtered. A water suspension of the filtrate was either sprayed or fed in sugar syrup to healthy bees. Heavy mortality showed up on the 6th and 7th days among the sprayed bees and on the 9th to 12th days in fed bees. Uninoculated bees lived beyond the 23rd day. Symptoms of paralysis - crawling, wing flutter, paralyzed hind legs (but not loose hair) - were shown by dying bees. Tests of the sample for insecticides and protozoan diseases were negative.

A spore-forming bacillus and a small coccus were isolated from bees of a colony showing paralysis at the Madison, Wis., laboratory. When the coccus was injected into adult bees mortalities resulted in 24 hours without septicemia symptoms. The dead bees contained large numbers of the coccus. This coccus was highly sensitive to streptomycin.

7. Mycosis. In feeding tests at Laramie, Wyo., spores of the mold Aspergillus repens, in sugar syrup were not pathogenic to bees. The mold may occur as a saprophytic on dead bees.

8. American Foulbrood. At Laramie, 16 colonies with light AFB infections were fed at 4 weekly intervals with the following medicants: Sugar syrup with 1 gm./gal. sulfathiazol; sugar syrup with 1 gm./gal. terramycin; powdered sugar with 7 gm. of sulfathiazol; and powdered sugar with 7 gm. of terramycin. These treatments greatly reduced the amount of infection in the colonies. However, none of them completely eliminated the disease in all colonies. Four control colonies fed only unmedicated sugar syrup developed severe infections of the AFB disease. The disease also spread to 3 additional colonies not in the test.

At Beltsville, Md., in vitro tests with Tylosin lactate at the 0.1 p.p.m. level inhibited growth of Bacillus larvae. In vivo tests of Tylosin lactate at the 1,000 p.p.m. level was nontoxic to adult honey bees. Material toxic to brood and adult honey bees was found in a comb received at Beltsville from Virginia. The identity of the toxicant was not established. Bees and brood exposed to the pollen or to acetone extracts of it were dead in less than 18 hours.

Thiamine (vitamin B₁) is an indispensable growth factor for Bacillus larvae. Therefore a study was made at Beltsville to determine the part thiamine may play in the host-pathogen relationship of Bacillus larvae and the honey bee. Thiamine was tested by direct infection into the thorax and abdomen of adult honey bees to determine the degree of toxicity for the honey bee. No apparent toxicity developed when injections were made to the level of 30 micrograms of thiamine per bee. Therefore, additional tests were performed by injecting spores of Bacillus larvae plus thiamine at a level of 30 micrograms per bee at concentrations of B. larvae spores of 1,000,000, 100,000 and 10,000 spores per bee. The course of the infection resulting in mortalities did not appear to be affected by the presence of this additional amount of thiamine. The tests were repeated using an increased dosage of thiamine at the extremely high level of 10,000 micrograms per bee. No effect was apparent on either the course of the infection or the degree of mortality when the spore concentration was 1,000,000 per bee. However, at the level of 100,000 spores per bee, the infection did not develop and the percentage of mortality was reduced. This finding raises the question as to whether excessive amounts of vitamin B₁ would be helpful in protecting the honey bee from infection by B. larvae spores at dosages less than overwhelming.

Six package colonies developed American foulbrood during September at Madison, Wis. This disease was associated with equipment from diseased colonies of last year. One heavily infected colony was burned, and all colonies in the yard were treated with sodium sulfathiazole. Treatment will be continued in the spring. The equipment and five colonies concerned have been isolated, and progress of treatment will be closely observed to determine the effectiveness of preventative use of the antibiotic.

9. European Foulbrood. Two attempts to produce EFB at Laramie, Wyo., by spraying unsealed brood in 9 colonies with a water suspension of naturally infected larvae resulted in the removal of 25% of the larvae, but only 4 EFB diseased larvae were observed and the disease did not spread to the rest of the brood.

At Laramie repeated gorging treatments with sugar syrup containing 4 gms./gallon of gallimycin cured 3 colonies with light EFB infections but not a colony that was heavily infected.

At Madison, Wis., outbreaks of EFB that commonly occur in May and June were prevented by April treatments, using 3 gorgings at 5 day intervals of syrup medicated with 100 mg. erythromycin activity or 150 mg. streptomycin per quart of syrup poured over the bees.

10. Spore-Forming Bacteria from Dead Brood. At Laramie, Wyo., larvae of three known age groups were inoculated individually, by contaminating the food in their brood cells with uniform sized droplets from a micrometer syringe with aqueous suspensions of the spores of one of three bacterial species isolated from dead honey bee larvae. Bacillus apiarius was recovered from only a single larva out of 600 inoculated with this species. B. pulvificiens was not recovered from any of 600, or B. laterosporus from any of 300, inoculated larvae. The percentage of larvae removed before sealing, both inoculated and control larvae to whose food sterile water was added, varied tremendously in different tests. The percentage removal of 2-to 3-day old larvae usually was greater than that of either younger (1-to 2-day) or older (3-to 4-day) larvae in alternate rows of the same brood combs, inoculated on the preceding or succeeding days.

11. Sac Brood Disease. A sample brood comb (from Nebraska) containing an unusually severe case of sac brood, with at least 54% of the sealed brood diseased, was placed in the center of the broodnest of a colony at Laramie, Wyo., on May 1. The bees gradually cleaned up the disease, which remained negligible in the colony throughout the summer. By contrast, a moderate sac brood infection in a local colony moved to the same apiary at first nearly disappeared, then recurred to a serious extent. Three gorgings at 1-week intervals with 1 gram chloromycetin per gallon of 50% sugar syrup had no effect on the sac brood infection. The sac brood decreased naturally during the latter part of the brood-rearing season.

Approximately 3,800 brood cells were individually inoculated at Laramie with filtrates of naturally infected sac brood larvae passed through filter paper, Mandler porcelain filters, or Millipore filters. Honey bee larvae of three different age groups were inoculated with dilutions of the filtrates. Significant amounts of sac brood (12 - 75%) developed in only four out of 22 inoculated combs. Each of these was inoculated with a Millipore filtrate, showing that this disease is caused by a virus. Most of the filtrate-inoculated larvae were completely removed. Many of the water-inoculated controls were almost completely removed also.

12. Nosema Disease. The bee attendants in only 3 of 100 caged queens shipped from Georgia to Madison, Wis., showed no Nosema infection. Infections of 50% or more of the bees per cage were found in a third of the shipment. In commercial package bee shipments from the Southern States 40 of 75 packages showed Nosema infection. This is about the usual degree of infection occurring in such shipments.

Bee feeding tests at Laramie, Wyo., with aqueous suspension of Nosema spores resulted in 81% to 95% infection. The same suspension irradiated with ultraviolet light produced only a 1 - 6% Nosema infection. After one month of refrigeration the irradiated suspension was fed to bees and 11 days later the colonies were inoculated with Nosema spores. There resulted only 23% infection in these bees. In contrast sister bees fed with unirradiated suspension after 8 days developed 52 - 67% infection. Colonies fed only unirradiated syrup developed 85% Nosema infections.

At Laramie, queens held in mailing cages at room temperatures (70 - 80° F.) for two and a half weeks showed 83% Nosema infection when dissected, whereas queens from the same commercial shipment held at 92° F. were only 39% infected. Their worker attendants averaged 80% and 39% Nosema infections at the two temperature conditions, respectively.

At Laramie, queens confined over glass microscope slides during recovery from carbon dioxide anesthesia excreted a yellowish fluid on the slides which was readily examined for Nosema spores. None were found. Further study is required to evaluate the usefulness of this technique for demonstrating Nosema disease in living queens.

At Laramie, an aqueous suspension containing both Nosema spores and Amoeba cysts was poured in a thin layer on a filter paper between two glass plates. An electric current from a 6-volt battery was passed through the thin film of water by copper wires attached to each end of the filter paper. Material migrating to the anode or cathode after periods of 4 to 24 hours was pipetted from the respective poles, mixed with 60% sugar sirup, and fed to caged healthy bees. Under the conditions of the test, the two protozoan species were not separated by the electric current. Suspensions from either pole infected the bees to a similar extent with both parasites.

Caged bees fed a mixed inoculum of Nosema spores and Amoeba cysts at Laramie, developed 87% Nosema, 1% mixed infection, and no Amoeba infection alone. Bees began dying with Nosema infections 8 days after inoculation. The Nosema-diseased bees showed 50% mortality on the 20th day whereas uninoculated sister bees did not show 50% mortality until the 56th day. Thus, Nosema disease reduced the average longevity of caged bees by about 5 weeks.

Routine surveys at Laramie of 20-or 30-bee samples per colony for Nosema and Amoeba diseases were made at irregular intervals. Average Nosema infections ranged from 0 to 19% in the spring, and from 0 to 40% in the summer, in different apiaries. In late autumn, Nosema disease was found in only two colonies in only one apiary, and in about 1% of the bees of the infected colonies. It is suspected that this exceptionally low Nosema incidence for this locality may be correlated with unusually warm, dry, autumn weather. Low infections (5 - 10%) of Amoeba disease were found in two colonies in February, one colony in June (together with Nosema), and only one colony in December.

At Madison, Wis., the severe winter of 1961-62 prevented bee flight and caused higher than usual levels of Nosema infection. Monthly samplings were made from all overwintering colonies December through April. Seven percent of the colonies showed Nosema infection in December. By April, 28% of the colonies were infected. Foci of heavy infection in the yards were associated with queenless colonies that were unable to rear brood.

At a conference on Nosema disease the Canadians reported control of Nosema with fumagillin (Fumidil B) administered in dust form. Tests with package bees at Madison showed only a slight reduction in disease incidence from this treatment, but three repeated applications at 3-or 4-day intervals gave a measure of control. Fumagillin feeding was effective in controlling Nosema in queen storage and queen rearing colonies and nuclei. In November 1962, all colonies were fed 0.9 gallon of heavy sugar syrup (67% sugar) containing 100 mg. fumagillin per gallon. This treatment effectively reduced the initial Nosema in these colonies through the January samplings. Light Nosema infections were found in only two colonies through the January samplings.

13. Alkali Bee Brood Disease. Alkali bees in artificial nesting sites near Riverton, Wyo., were found to have a serious brood disease, even though mite infestations had been eliminated. No bacteria were evident in the diseased brood. At Laramie, it was suspected that the disease might be a virus infection similar to sac brood disease of the honey bee. Accordingly, a filter paper filtrate of diseased alkali bee brood macerated in water was used to inoculate individual brood cells containing 1 - 2-day old honey bee larvae. No disease appeared, and 97% of the inoculated brood developed into healthy bees, equivalent to that in water-inoculated controls. The virus may be specific to the alkali bee host Nomia melanderi.

14. Anthophora Bee Brood Disease. A single clay-like subterranean brood cell containing a diseased Anthophora bee pupa was sent to Laramie from Logan, Utah. Stained smears of the bee tissues failed to reveal any microorganisms.

C. Bee Behavior

At Madison, Wis., a reaction expressed by stopping a body activity or movement was identified in honey bees at a sound frequency range between 200 c.p.s. and 1,200 c.p.s. An optimum frequency of 960 c.p.s. was indicated. Amputation of the tarsi of pairs of legs and subsequent sound stimulation of the bee indicated that the forelegs are most important as a sound receptor or transmitter to the sensitive organ. Whether the sensitive organ is the scolopophorous organ or organs of the leg was not precisely determined. Leg contact with the substrate is an absolute necessity for sound reactions.

Research has been resumed at Baton Rouge, La., to find attractants in pollen. Most of the work to date has duplicated that which was originally done eight years ago. The following new information has been obtained: The attractive fraction in pollen is neutral, rather nonpolar, resistant to heat and ultraviolet light, acid and base stable for short periods, and deteriorates within 3 weeks in storage. Odor does not seem to be an attractant.

At Logan, Utah, in an attempt to determine the effect of stored-pollen reserves on pollen collection in confinement, only in a greenhouse cage did a pollen-poor colony collect pulverized pollen outside its hive. The pollen-rich colony showed no interest in collecting pollen under the same conditions. When the colonies were subsequently placed in a greenhouse cage, both eventually collected pollen; the pollen-poor one first and more avidly. Three colonies later brought into the bee room in February at Logan commenced pollen collection readily. It is postulated that in addition to pollen reserves, the duration of the dormant period before the bees were activated inside may have influenced the readiness of the bees to collect pollen.

At Beltsville, Md., for a period of approximately 6 weeks, an apparently normal queen laid a large percentage of multiple eggs in many brood cells. The multiple larvae were not removed after hatching but were tolerated in many instances through the mass feeding. When 3, 4, or 5 larvae were present per cell, these were generally reduced to 2 larvae which were tolerated until after the mass feeding period. Some of these larvae attained a weight of over 20 milligrams before removal from the cells. The surviving larvae in all instances went on to maturity and eventual emergence.

At Beltsville, an apparatus for extracting honey bee scent consisted of a closed chamber which would hold 4 pint mason jars and had a port through which a continuous stream of air could be introduced plus an exhaust port from which the air and the collected scent material could be exhausted. Approximately 500 honey bees were placed in this apparatus daily for a period of 5 weeks. The air recovered from this closed apparatus was passed

over a drying trap to remove moisture. The scent material obtained was then condensed on aluminum filings buried in dry ice. After each 5 days' extraction the condensed material was recovered from the aluminum filings by washing them with distilled acetone. Over 12,000 bees were exposed in this apparatus. The extracted material was turned over to the Pesticide Chemicals Research Branch for concentration and possible identification.

D. Utilization of Honey Bees and Other Insects in Crop Pollination

1. Sweetclover. At Madison, Wis., seed increase studies in cages with low coumarin sweetclover were carried out under 3 types of screen cover - 20-mesh saran cloth and 8- and 12-mesh fiberglass. The areas of the cages were 39,000 and 5,000 sq. ft. for saran cloth and 34,000 sq. ft. for fiberglass screen. Although bees worked well in all the cages, counts of bee visitors indicated the seed yields may range from high to low under 8-, 12- and 20-mesh in that order. Light reduction by the screen was apparently an important factor.

At Baton Rouge, La., a species of wild bee of the Halictine group has been identified as Lasioglossum versatum R. by Dr. Michener, University of Kansas. This bee is nesting in the ground on the campus of Louisiana State University and superficially appears to be the principal insect pollinator in the strawberry belt in Louisiana.

2. White Clover. At Baton Rouge, twenty colonies of honey bees were provided to pollinate from 20 to 25 acres of white clover (Trifolium repens) at the Idlewild Experiment Station. In earlier years there were no known white clover plants in pastures adjoining the station, but in 1963 some pastures within flight range of the colonies contained clover. Seed production continued to be unsatisfactory even though bee visits to the clover blossoms seemed normal. More than one half of the pollen collected in a pollen trap consisted of white clover pollen, showing that bees were visiting the clover blossoms. There were indications that trace elements (boron, copper, molybdenum, zinc) which had been applied to the soil by the experiment station caused an increase in the number of fertile florets and seeds per clover head. It is possible that pollination is adequate at Idlewild, but that soil conditions interfere with maximum seed production by the plant.

3. Cotton. Two Arizona Pima S-2 cottonfields were contracted for and supplied with bees for pollination in 1962. One field contained 100 acres and received 150 colonies of honey bees in 12 locations. The other contained 180 acres and was supplied with 180 colonies in 25 locations around the field.

At Tucson, data were taken from the 100-acre field. Over a 2-month period the average colony gain was only 34 pounds. The bee visitation to cotton was not satisfactory. Apparently the Pima S-2 cotton is less attractive to bees than the Pima S-1 variety previously worked with.

4. Safflower. At Tucson, an experiment was conducted in cooperation with Dr. Dave Rubis of the University of Arizona Agronomy Department to determine the effect of bees on the seed yields of two varieties of safflower. One was the normal commercial strain, Gila, while the other was a thin-hull selection developed by Dr. Rubis. The results showed that Gila safflower is capable of setting seed in the absence of bees. The thin-hull selection, because most of the anthers dehisce after stigma extrusion is complete, requires visits from bees to distribute the small amount of pollen that escapes the stigmal sheath. Safflower is highly attractive to bees; it is a good pollen source; and it produces a fair honey crop. The honey is thin, dark amber in color, and has a flavor that is not too desirable. The honey does not granulate even when left in the hive for a year. The nectar sugar concentration is about 20% as taken from nectar sacks of bees.

Bees visit safflower for nectar most of the day. Peak visitation is between 9 - 11 a.m. Pollen collection is mostly in the early morning. The Gila variety yields considerable quantities of pollen. Both Gila and thin-hull varieties are attractive to honey bees. Bees with pollen (presumably from the Gila variety) were observed visiting the thin-hull for nectar in Tucson.

At Tucson, the exclusion of bees in cages over the safflower plants reduced the seed yield by 50% in the thin-hull variety, but only 2.5% in the Gila, in comparison with the yield in cages containing bees. Caging (with bees) reduced the Gila variety yield by 15% over the yield of open-pollinated plots, but the reduction was slight with the thin-hull variety.

At Tucson, the oil content of seeds from the plots caged with bees, exceeded those of open plots by 2.1% with Gila, and 0.8% with the thin-hull variety. The increase in Gila would amount to 50 lbs. of oil/acre, which would be sufficient to make bees of value to the commercial producer for the benefit of oil content alone.

5. Red Clover. At Logan, Utah, observations were made on the pollinators visiting two experimental plantings of tetraploid and diploid red clover. Where the planting was isolated from colonies of honey bees and was surrounded by uncultivated land, bumble bees and species of Osmia were the most abundant pollinators. Many other pollinators were present but only in small numbers. Where the planting was not isolated from honey bees, these bees were the most abundant pollinators, but bumble bees, because of their greater efficiency, were probably nearly as important. Where diploids and tetraploids were side by side, yields were lower and differences in seeds per head between the varieties were not significant, perhaps because of complications caused by triploidy.

6. Alfalfa. At Logan, a total of 356 colonies from five apiaries in Box Elder and Cache Counties were checked for percentage of alfalfa pollen carried into the entrance. The sampling device developed for the purpose consisted of a motor and squirrel cage blower for suction connected with a

piece of vacuum tubing leading to a plastic centrifuge tube into which the bees arriving at the hive entrance were sucked. In the field a 12-volt automobile battery was used as a power source.

After checking all colonies on three dates, the five highest and five lowest colonies in terms of alfalfa pollen collection were selected and sent to the Baton Rouge, La., laboratory. Five or more queens bred from each stock (selected primarily on the basis of egg viability) at Baton Rouge will be sent back to Logan in the spring. From colonies containing these queens, the highest and lowest alfalfa-pollen-collecting colonies will again be selected and sent to Baton Rouge for further testing next year. It should be possible by this means to determine whether there is a genetic basis for alfalfa pollen collection.

7. Lipids in the Bee Diet. At Tucson, Ariz., the total and percent saponifiable and unsaponifiable lipids were determined in adult bees that had consumed diets of known fat content and compared with those of newly emerged bees not allowed to feed and older hive bees on their natural diets. The total lipid content did not differ significantly in bees fed fat-extracted and unextracted cottonwood pollen diets and was about equal to the total lipid content of bees fed sucrose candy. Bees fed unextracted dandelion pollen had a significantly higher total lipid content than those consuming other diets. Total lipids extracted from newly emerged bees had the highest percent of saponifiable material and the lowest amount of unsaponifiable substances. Lipids of bees fed sucrose candy had the highest percent of unsaponifiable material. The total lipid extracts of all bees analyzed without the digestive tract contained considerably more saponifiable than unsaponifiable material.

8. Wild Bee Pollinators. Taxonomic and biological studies of Anthidium (Hymenoptera, Megachilidae) were continued in 1962 and illustrations were completed for 27 species at Logan, Utah. Although in the same subgenus (Eutricharaea) and almost identical in size, shape, and color, M. rotundata and M. concinna are not difficult to distinguish in either sex. In the female the apical tergite of rotundata is more accurate in profile and has more erect pubescence. The male of rotundata has the teeth on the carina of the apical tergite mostly concentrated on an irregular, emarginate, median prominence, whereas in concinna the teeth are well separated and distributed around the carina, which is not particularly prominent medially. Also, the coxopodites of the male genitalia of rotundata have an apical process which is missing in concinna. The two species can also be distinguished by their leaf cells and cocoons. The leaf cells of concinna are more deeply telescoped into each other than are those of rotundata and the leaf pieces are more elongate and rectangular. The cocoons of rotundata are longer than those of concinna and the anterior end has the fecal pellets closer to the summit.

At Logan, observations were made on the nesting activity of M. rotundata including the effect of nesting materials, types of nests, straw sizes, color, movement of nests and temperature and humidity. Biology and ecology studies of a tomato pollinator, Anthophora occidentalis, demonstrated that the nesting season is primarily in July, that egg and larval development take about 15 days, that nesting occurs in vertical clay banks or walls, that thistle is the preferred host, and that tomato is quite attractive.

E. Effect of Pesticides on Honey Bees

Observations were made August 21-22 near Buckeye, Ariz., on the effectiveness of confining commercial apiaries under wet burlap tarpaulins to protect them, in this case, from methyl parathion and endrin sprayed by aircraft on 300 acres of nearby cottonfields. Colonies confined until 3 p.m., 4 hours after the applications were completed, suffered no mortality or apparent distress during the hours of confinement even though external air temperatures reached 104° F. Colonies left uncovered showed heavy mortality by 10 a.m. Within an hour after the bees were released to forage from the hives which had been covered they began to die in front of the hives and heavy mortality resulted. The bees were effectively protected during the period of confinement but not from the residual action of the insecticides. Length of time of confinement necessary for complete protection is unknown. It probably varies with different pesticides.

At Beltsville, Md., tests for toxicity to honey bees of solutions of various concentrations of new insecticides yielded the following results: ENT-25827, ENT-25828, ENT-25801, ENT-25831, ENT-25833, Bayer 47416, and Hooker HRS-1296, were less toxic than DDT to the honey bee; ENT-25819, ENT-25835, ENT-25788, Zectran, and ENT-28532 were more toxic than DDT but less toxic than malathion to the honey bee; ENT-25826, ENT-25923, ENT-25960, and ENT-25834 were more toxic than malathion to the honey bee.

At Beltsville, honey bees killed by various insecticides were extracted with organic solvents and the solutions examined under ultraviolet light after being spotted on filter papers. A definitely different pattern was revealed by the extracts from bees killed by chlorinated hydrocarbons, phosphates, or carbamates. However, this differentiation was true only when the bees were killed by an excessive amount of insecticide. When the amounts of the insecticides were reduced to an absolute minimum for mortality, differences were not obtained. This technique may prove of value for detecting honey bees killed by insecticides.

At Logan, Utah, bees from small observation colonies were trained to collect Sevin containing syrup from a feeder 25 to 30 yards from their hives. Individual marked bees made from 2 to 7 trips before becoming so affected that they no longer flew from the hive or were lost and presumed dead. They carried .08 to 1.6 µg. Sevin in syrup containing blue food color. Although the carrier bees usually recovered, hive bees receiving the syrup were often

killed. Bees trained to take untreated syrup while resting on a Sevin-treated surface made 12 to 59 trips before becoming seriously affected. Three bees exposed to residues of 1 pound per acre of Sevin recovered. One bee died and one recovered after exposure to 2-pounds-per-acre residues.

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INSECT PHYSIOLOGY AND MODE OF ACTION OF INSECTICIDES
AND THEIR METABOLITES

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Problem. Basic research in insect physiology is essential to the development of more efficient insecticides and new approaches to insect control. The increasing development of resistance to insecticides by insects has emphasized the need for additional information on the mode of action and metabolism of insecticides in insects and the mechanisms of the resistance to insecticides. More knowledge is also needed on the normal physiology and biochemistry of insects to permit a comparison and interpretation of the data obtained from studies on insect toxicology. Basic research in insect biochemistry and physiology, including insect nutrition, will provide a better understanding of the biochemical and physiological systems which regulate insect growth, metamorphosis, reproduction, and diapause, and the chemistry and action of the hormones which mediate these systems. Knowledge gained from such research is essential to the development of new methods of effective insect control which are safer and more selective in their action than the methods now being used. More basic information on the response of insects to light, sound, food, and sex attractants could contribute to better insect control. Insects are useful test animals for basic physiological studies on life processes.

USDA PROGRAM

The Department has a continuing long-term program involving insect physiologists, biologists and chemists engaged in basic studies in insect physiology and biochemistry and in the mode of action of insecticides and their metabolites. At the Pioneering Research Laboratory on Insect Physiology at Beltsville, Md., basic research is conducted on biochemistry and physiology of lipids in insects, insect nutrition and hormones, effect of light on insect growth and development, and effect of chemical carcinogens on insects.

The Federal scientific effort devoted to research in this area totals 10 professional man-years. Of this number 3.9 is devoted to the biochemistry and physiology of lipids in insects, 3.9 to insect nutrition and hormones, 2.0 to effect of light on insect growth and development, and 0.2 to effect of chemical carcinogens on insects.

Additional research in this area is provided by the following P. L. 480 projects: S5-ENT-3 Colombia (2 professional man-years); A7-ENT-6 India (2 professional man-years); E21-ENT-3 Poland (1 professional man-year); and E21-ENT-4 Poland (1 professional man-year).

A research contract with the Mississippi State University, No. 12-14-100-6895(33), for studies on experimentally induced insecticide resistance in a vertebrate, was executed June 24, 1963.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Biochemistry and Physiology of Lipids in Insects

1. Major Sterol from Cholesterol Fed American Cockroaches. In a previous study at this laboratory concerned with the fate and distribution of 4-C¹⁴-cholesterol in the male American cockroach, it was found that nearly all of the radioactive sterol fraction behaved like unchanged cholesterol when analyzed by gas-liquid chromatography and reverse isotope dilution. However, other workers have reported that this insect converts 4-C¹⁴-cholesterol to its "major natural sterol," a compound of unknown structure. This sterol was reported to differ from cholesterol in its chromatographic behavior in that it was slightly less polar.

To resolve these differences, the sterols (0.475 gm.) were isolated from adult male American cockroaches fed for 20 days on a synthetic diet containing 0.1% cholesterol. Analysis of the crude roach sterol by several gas-liquid chromatographic systems showed the presence of a single compound with the same relative retention time as cholesterol. Following purification through the dibromide the roach sterol and its acetate corresponded to cholesterol and its acetate in melting point and optical rotation. In addition, the optical rotatory dispersion curves, infrared and nuclear magnetic resonance spectra, and relative retention times by four gas-liquid chromatographic systems were also identical, demonstrating that unchanged cholesterol was the major sterol present in these insects. This research was conducted in cooperation with the Laboratory of Chemistry of Natural Products, National Institutes of Health, Bethesda, Md.

2. Cholesterol Turnover in Adult American Cockroach. In order to determine whether cholesterol is metabolically active or merely stored in the tissues of the American cockroach, Periplaneta americana, the rate of appearance of C¹⁴ labeled cholesterol in the tissues of the insect was determined over a period of 60 days. Newly emerged adult male insects were placed on a semi-defined diet containing 4-C¹⁴-cholesterol. Every 10 days, representative groups of insects were selected, the alimentary tract removed from each insect, and the insects were then saponified. The unsaponifiable lipids were chromatographed on alumina and the specific activity of the tissue cholesterol was determined. The change in specific activity over the period of time was logarithmic, and when analyzed kinetically, it followed a first order reaction pattern. The half-life of the cholesterol in the tissues was calculated to be 82 days. This evidence suggests that the tissue cholesterol is constantly being renewed from the diet and that the cholesterol is being metabolized and/or excreted intact. Studies are currently being undertaken to determine if the cholesterol molecule is being modified by the insect.

3. Identity of House Fly Sterol. Preliminary studies have shown that the major sterol from unfed adult house flies reared by the CSMA procedure is not cholesterol. Other workers have reported that three sterols are present in the adults and eggs of house flies reared by the CSMA procedure. These workers designated the major sterol as "Muscasterol" and believed it to differ from cholesterol beyond C-22 on the side-chain. They further speculated that the precursor of the fly sterol could be a phytosterol which was dealkylated in the fermenting media and concentrated by the fly or dealkylated directly in the fly. However, studies in this laboratory have shown that house flies reared aseptically on a semidefined diet containing β -sitosterol (a major sterol of CSMA media) do not alter its side-chain. Thus it appeared desirable to isolate, identify, and determine the origin of the house fly sterol.

The sterols were isolated from the unsaponifiable fraction of house flies by column chromatography and digitonin precipitation. This material showed four distinct peaks when analyzed by gas-liquid chromatography (GLC). The components in order of increasing retention time were present in relative quantities of 2.8, 1.5, 74.3, and 21.4%, respectively. Neither the major sterol nor its derivatives could be separated from the fourth component (β -sitosterol) by various adsorption chromatographic systems or by crystallization from methanol.

However, a commercial source of β -sitosterol of 69% purity contained a sterol contaminant with the same relative retention time as that of the major house fly sterol. This sterol could be concentrated to 91% purity (GLC analysis) by repeated fractional recrystallization from acetone (10 times) at room temperature. Further purification through its 3,5-dinitrobenzoate yielded a compound with a final purity of 99%.

The major house fly sterol was purified under similar conditions and analyzed to be 94% pure (GLC analysis, 6% β -sitosterol). The infrared spectra of the house fly sterol and its ester derivatives were identical with that of the sterol derived from the commercial sample of β -sitosterol and its respective derivatives. The physical properties and infrared spectra of both sterols and their derivatives characterized these sterols as campesterol (α -methyl at C-24). The physical properties of campesterol and its derivatives were in complete agreement with those in the literature.

The secured identity of the house fly sterol as campesterol suggested that it was derived from the CSMA media. Yet, gas-liquid chromatography of the unsaponifiable fraction of the CSMA media showed β -sitosterol to be the major sterol, with campesterol present in a lesser amount. The possibility that the smaller size particles being consumed by the house fly were richer in campesterol was readily eliminated by experimental results which indicated that a high percentage of campesterol was present in the larger sized particles of the medium.

4. Fate of C¹⁴-Cholesterol in House Fly Larvae. Cholesterol, which has been reported to serve as a sparing sterol in several insects, has also been found to fulfill this same function in the house fly, *Musca domestica*. To ascertain the fate of this sparing sterol in this insect, house flies were reared aseptically to pupation on a semidefined diet containing 0.2% C¹⁴-cholesterol and subminimal quantities of cholesterol. Analysis of the total lipid extracts from the pupae indicated the presence of 14.5 µg. equiv. of C¹⁴-sterol per insect. Column chromatographic analyses of the extracts on alumina showed that greater than 94% of the C¹⁴ compounds present were as free sterols and only 4.1% as sterol esters. This predominance of free sterols differs from previous findings with the German cockroach reared under sparing conditions with C¹⁴-cholesterol, where about 45% of the total C¹⁴ compounds were found to be esterified. Further chromatographic analyses of the free sterols as their acetyl derivatives indicated a single major peak representing 99.1% of the radioactivity. In addition, two minor more polar peaks were also detected.

When an aliquot of the major peak was analyzed by reverse isotope dilution with authentic cholesterol, the radioactivity was all lost by the sixth recrystallization, indicating no conversion of cholesterol to cholesterol. Reverse isotope dilution of another aliquot of this fraction with cholesterol, by purification via the Anderson-Nabenhauer technique, and oxidation to cholesterolone, indicated that greater than 97% of the radioactivity represented unchanged cholesterol. These findings were confirmed by gas-liquid chromatographic analyses. These results point to a major difference in the metabolism of cholesterol during its utilization as a sparing sterol by the house fly and the German cockroach. The latter insect converts about one-half of the cholesterol to Δ^7 -cholesterol, whereas in the house fly pupae nearly all of the C¹⁴ compounds present represented unchanged cholesterol. Studies are currently underway to determine the fate of cholesterol in the adult fly.

Results of this research have been presented in the following papers prepared for publication: Thompson, J. J., Louloudes, S. J., Robbins, W. E., Watters, J. A., Steele, J. A., and Mosettig, E. The identity of the major sterol from house flies reared by the CSMA procedure. Accepted by Jour. Insect Physiol.

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B. Insect Nutrition and Hormones

1. Modification of Tenebrio Test for Assay of Juvenile Hormone Activity.

During the course of studies on insect hormones a variation of the "Tenebrio test" for juvenile hormone activity was developed which permitted quantitation of the results, and which relied upon the retention of clearly defined pupal characters in the adult tenebrionid beetle following injection of an active material. The characters utilized were more easily and positively determined to be the result of juvenilization than the previous method of using unpigmented patches of "pupal" cuticle as the sole criterion of activity. The characters most sensitive to juvenilization were the lateral abdominal gin traps and the urogomphi. The gin traps are sclerotized structures projecting laterally from each abdominal segment and the urogomphi are sharp pointed sclerotized projections located at the end of the larval and pupal abdomens. These structures have no external counterpart in the adult except in the male beetle which retains a very small remnant of the urogomphi. These structures were retained in the adult to a greater or lesser degree depending upon the activity of the dose of the juvenilizing compound. The greatest possible effect was complete repupation and this was achieved with some of the materials investigated, such as farnesyl methyl ether which causes repupation with a dosage as low as 5 micrograms.

The outstanding effect of these compounds is the deformation of the genitalia, a situation which if serious enough would preclude copulation and hence reproduction. So far only the gross external morphological effects have been observed; investigation of effects on internal structures which may be even more sensitive is currently under study.

2. Juvenile Hormone Activity of Isoprenoid and Straight-Chain Alcohols and Derivatives. Using the above bioassay, studies on the juvenilizing activity of various isoprenoid compounds have delineated some of the structural features which influence this activity.

Reduction of farnesol to the saturated alcohol resulted in decreased but measurable activity. However, there was a complete loss of activity following removal of the hydroxyl group and reduction to the saturated hydrocarbon.

Conversion of farnesol to its methyl ether increased its activity several hundred fold, but methylation of the tertiary hydroxyl of nerolidol did not increase its activity, which emphasized the importance of the primary hydroxyl for activity. The methyl ether may be more active because of greater chemical or biochemical stability. Farnesyl acetate and farnesyl trimethyl acetate were nearly as active as the free alcohol, whereas farnesyl oleate showed little activity.

In addition to the numerous isoprenoid alcohols and their derivatives, certain straight-chain saturated alcohols from C-8 to C-14 were active, which suggests that the isoprenoid alcohols are not unique in possessing juvenile hormone activity.

Straight-chain saturated alcohols from octanol to tetradecanol exhibited low activity. With the exception of octanyl methyl ether, all of the methyl ethers possessed enhanced activity and dodecanyl and tetradecanyl methyl ethers were particularly active. The greater activity of the dodecanyl derivative is interesting in view of the fact that the carbon skeleton determining the chain length of farnesol is a 12 carbon unit. While dodecanyl methyl ether was slightly more active than farnesol, it was considerably less so than farnesyl methyl ether, suggesting that perhaps unsaturation or branching may play a major role in the degree of juvenilization. Such investigation is being pursued.

In spite of the very high order of juvenile hormone activity for farnesol and derivatives, it is unlikely that these are actually the natural hormone as they do not behave chromatographically like the active materials from *Cecropia* or the tobacco hornworm.

3. Juvenile and Gonadotropic Hormone Activity of Insect Extracts. Previous workers have demonstrated juvenile hormone activity in a wide variety of animals, plants, and microorganisms. This laboratory has demonstrated the gonadotropic action of an extract of the male *Cecropia* moth in allatectomized American cockroaches. When this extract was chromatographed on a column of silicic acid, the fraction containing the gonadotropic activity also showed juvenile hormone activity when tested by the above modification of the *Tenebrio* test. Rechromatography of this fraction on other adsorbents failed to separate the two activities, indicating that the hormones involved are either the same or very similar compounds.

Further investigations of crude and fractionated lipid extracts of other more common insects have revealed the presence of detectable amounts of juvenile hormone activity in a number of species. Some insects which have shown this activity are: tobacco hornworm larvae, pupae and adults, wax moth larvae, and adult males of two species of cockroaches, (*Nauphoeta cinerea* and *Blaberus craniifer*), and the silkworm moth.

Column chromatography of the crude lipids of the tobacco hornworm has shown that the juvenile and gonadotropic hormone activities are eluted together in the same fraction as the corresponding activities in *Cecropia* extracts. The data indicate that the hormone(s) may be the same or very similar in these two species.

4. Food Attractants and Stimulants of Alfalfa Weevil and Silkworm.

Feeding attractants and stimulants have been reported to play a major role in host plant recognition and specificity in many phytophagous insects. The isolation and characterization of the attractants and stimulants would permit the role of the individual chemicals to be correlated as to their effect on the total feeding behavior of the insect, i.e., orientation, biting response, and sustained feeding.

Alfalfa weevil and silkworm larvae respond to ether extracts of alfalfa and mulberry, respectively, by oriented movements toward its source of the extracts. Long distance effects were not observed with either insect. Fractionation of the mulberry extract by column chromatography gave three different active fractions in that the silkworm larvae responded to them by orientation and/or by biting reactions. There were no reciprocal responses, indicating that the insects respond or react only to substances present in their normal host plants. Further purification of the active components is underway.

Feeding stimulants are distinguishable from feeding attractants in that they are perceived by the insect upon contact with the substrate, presumably through taste receptors located in the mouthparts. Several substances have been shown to act as feeding stimulants, particularly the simple sugars. However, feeding stimulants present in plants which presumably contain sustained feeding have been hypothesized to exert this stimulation in a synergistic manner in the presence of other stimulating substances. The adults of the alfalfa weevil will feed on an artificial diet containing water soluble substances extracted from alfalfa. They will not, however, feed on such diets in the absence of sugars. An assay technique based on this behavior of the weevil was used to extract and purify by fractionation the feeding stimulant from alfalfa. An organosoluble derivative of the stimulant has been prepared from a purified extract, which upon regeneration still is active. Work on the further purification and characterization of this stimulant is in progress. This study was conducted in cooperation with the Grain and Forage Insects Research Branch.

C. Effects of Light on Insect Growth and Development.

1. Responses of House Flies to Light. Although vitamin A has been considered unnecessary in insect diet, there is some evidence that insects have the same retinene visual pigments as mammalian rods.

House flies were reared on a vitamin A-free diet without contamination by microorganisms. These flies were compared with flies reared on the CSMA media which contained fermenting plant materials rich in carotene. Neither the aldehyde (retinene₁) nor the alcohol (retinol₁) of vitamin A was found in the heads or the eggs of flies reared on the vitamin A-free diet. Improved procedures detected both in heads of CSMA flies. This discovery of retinol in an insect supports some role for vitamin A in insect vision.

With a simple device, movement of these flies toward or from light was measured in 25 mμ increments from 300 to 725 mμ. Both diets produced photosensitive flies. Flies on the vitamin A-free diet were attracted to wavelengths from 375 to 675 mμ; CSMA flies moved away from 525, 550, and 575 mμ. The failure of flies depleted in vitamin A to be repelled by "green" light was not due to reduced intensity perception. At lowered intensity the response was slower but unchanged. Nevertheless, a thousand times more light was required for vitamin A-depleted flies to give the same optic nerve response as CSMA flies. The responses of larvae differed from adults and varied with age. Removing wings did not alter the response of adults. More females than male adults moved toward light and newly emerged flies were most photopositive. In CSMA flies, a photonegative response to 550 mμ developed two days after emergence. Experiments indicated competitive dietary effects. Something in plant material (bran, alfalfa, and yeast) when fed to larvae produced adults that were photonegative to 525-575 mμ light. β-carotene had no effect. The effects of dietary components on behavior toward light, on electroretinograms, and on histology of the eye are being investigated.

Some of the results of this study have been accepted for publication:

Cohen, Charles F. and Barker, Roy J. The vitamin A content and spectral response of house flies reared on diets with and without a vitamin A source. Accepted for publication in Jour. Cellular and Comparative Physiology.

D. Effect of Chemical Carcinogens on Insects

1. Effect of Certain Carcinogenic 2-Fluorenamine Derivatives on House Fly Larvae. Tumors, natural or induced, have been reported to occur in representatives from most of the major invertebrate phyla. In insects the best known and most intensively studied neoplasms are the hereditary tumors of Drosophila melanogaster. Although the incidence of these hereditary tumors may be increased with certain vertebrate carcinogens, attempts to chemically induce tumors in non-tumorous strains of insects have generally been unsuccessful.

To study the effect of vertebrate carcinogens on growth and development in the house fly, house fly larvae were reared aseptically on a semidefined diet containing certain representative vertebrate carcinogens. The carcinogenic compounds were coated on the dry components of the diet with a volatile organic solvent. Thirteen compounds known to produce tumors in vertebrates were tested. These included aromatic hydrocarbons, aromatic amines including four 2-fluorenamine derivatives, an azo dye, and a carbamate.

All of the 2-fluorenamine derivatives tested profoundly affected larval development when added to the diet at concentrations ranging from 0.002% to 0.04% (wet weight). In order of increasing effectiveness these compounds were: 2,7-fluorenediamine, N-2-fluorenyl-acetamide, 2-acetamide-7-fluoro-fluorene, and N-hydroxy-N-2-fluorenyl-acetamide. At higher concentrations these compounds were toxic to the newly hatched larvae and at intermediate and lower concentrations they were severely inhibitory to growth, development, and pupation. In addition, at certain concentrations, only a low percentage of test insects that pupated emerged as adults. Examination of last instar larvae reared on diets containing these carcinogens revealed many with gross malformations and darkened or "melanotic" areas. The pupae exhibited annular constrictions, abnormal protrusions, and imperfectly sclerotized areas.

Two structurally related compounds, fluorene and 9-fluorenone, which have been reported to be noncarcinogenic, were tested for comparison. 9-Fluorenone was nontoxic and fluorene was toxic only at the maximum concentration (0.04%). Otherwise, at the concentrations tested these two compounds neither inhibited growth, pupation, or adult emergence, nor produced the abnormalities observed with the carcinogenic 2-fluorenamine derivatives.

When a histopathological examination was made of the malformed larvae and pupae, reared on a diet containing 0.004% N-hydroxy-N-2-fluorenylacetamide, tumors were observed which were similar to hereditary tumors of Drosophila as described in the literature.

The carcinogenic action of the isomeric fluorenylamines and derivatives has been extensively studied in vertebrates and use of these compounds has greatly expanded our understanding of chemical carcinogenesis. These compounds also appear to offer a means for studying chemically induced tumors in the house fly and as such should add appreciably to knowledge of insect tumors and comparative oncology. This research was conducted in cooperation with the Insect Pathology Laboratory.

Results of this research have been presented in the following paper:

Shortino, T. J., Cantwell, G. E. and Robbins, W. E. Effect of certain carcinogenic 2-fluorenamine derivatives on house fly larvae (Musca domestica L.). Submitted to the Jour. Insect Pathology.

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Barker, Roy J., Mayer, Ann, and Cohen, C. F. 1963. Photoperiod effects in Pieris rapae L. Ann. Entomol. Soc. Amer. 56: 292-294.

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